A MANUAL

FOR THE

Clinical Laboratory

AN INTRODUCTION

INTO THE

LABORATORY METHODS OF CLINICAL PATHOLOGY

BY

EDWARD L. WHITNEY, M. D.

DEMONSTRATOR OF CLINICAL PATHOLOGY

AT THE

BALTIMORE MEDICAL COLLEGE

PUBLISHED BY THE AUTHOR

1896.

Press of
C. W. Schneidereith & Sons,
Baltimore.

PREFACE.

The friendly interest which has been shown by many colleagues and on part of a large number of Students in our special course on Diseases of the stomach and intestines have caused me to urge upon the author of this concise and practical little book—its speedy publication. Dr. Edward L. Whitney is eminently qualified for adapting this compend to the needs of the student and practitioner alike. His experience as a laboratory instructor and hospital physician has guided him admirably to present in a condensed and concise form most of the essential and indispensable aids to Clinical Diagnosis that are offered by chemistry, bacteriology and the technics of the microscope.

I hope it may stimulate its readers to further study of the literature of the modern methods of Clinical Diagnosis, which is the main hope of all true Asclepiads, for taking Diagnosis and Treatment entirely out of empiricism and founding it upon ascertained facts. May this little volume by Dr. Whitney find its way to the studies of many practitioners and students.

JOHN C. HEMMETER, M. B., M. D., Ph. D.

URINALYSIS.

AMOUNT IN TWENTY-FOUR HOURS.

The amount of urine excreted in a day depends upon a number of factors. In general we may say that the average adult male under average conditions excretes about 1500 cubic centimetres varying from 1500 to 2000 cubic centimetres per day. The female excretes a smaller amount varying from 1200 to 1500 cubic centimetres per day.

The amount of urine is increased:

1. When an increased amount of fluid is ingested as in beer drinkers.
2. When the amount of fluid lost by perspiration is small.
3. In the so-called phosphatic diabetes.
4. In hysteria.
5. In certain of the neuroses.
6. In diabetes insipidus.
7. In diabetes mellitus.
8. In certain forms of chronic nephritis, (especially the small granular kidney.)

The amount of urine is decreased:

1. When the amount of fluid ingested is small.
2. When perspiration is profuse.
3. In acute nephritis and the large white kidney.
4. In most of the acute fevers.
5. When a large amount of fluid is withdrawn from the system through some other channel as in diarrhoea and dysentery.
6. When there is an exudation going on in the body as exudative pleurisy, ascites, etc.

COLOR.

The average color of normal urine of specific gravity 1020 and 1500 cubic centimetres volume for twenty-four hours is straw or wine yellow, or a light amber color. It is subject to considerable variation during the day, that passed in the morning being usually the highest colored. Even under normal circumstances the urine varies from a pale watery fluid to a highly colored reddish brown liquid. The pale watery urine is usually of a low specific gravity and contains a small amount of coloring matters as well as urea and salts. Highly colored urine is, on the contrary, usually of a high specific gravity, strongly acid, and it

contains relatively large amounts of coloring matters, urea and salts. Pathologically the urine is subject to very great variations of color. Abnormally light colored urine is often found in diabetes insipidus and diabete mellitus, in hysteria and in convulsions, in all of which there is usually polyuria and the deficiency in color is due to the dilution of the coloring matters. Light colored urine also is found in diseases of the kidneys which increase the amount of urine and at the same time decrease the amount of coloring matters excreted. This is most frequently found in chronic interstitial nephritis (small granular kindney) an in some cases of amyloid degeneration of the kidneys.

Highly colored urine, approaching red, is most often induced by the acute fevers. This is due in part to their concentration and in part to the occurrence of abnormal coloring matters.

ODOR.

The odor of freshly voided urine is peculiar and slightly aromatic. The odor of the more concentrated urines is the most pronounced. If the urine becomes alkaline from standing and fermentation taking place the odor becomes peculiarly repulsive and the odor of ammonia becomes very plainly perceptible. The ammonia is due to the decomposition of urea with the formation of ammonium carbonate and the putrescent odor is due to the decomposition of mucin and other organic material. In diabetes the urine often has an odor which has been likened to that of hay, by others it is likened to that of violets. In cystitis with alkaline fermentation of the urine, the urine often has the unpleasant ammoniacal odor when passed.

Certain drugs impart their odor to the urine to a marked degree, the most common of these being cubebs, copaiba and sandal-wood.

TRANSPARENCY.

Normal human urine is clear when passed, soon however showing a faint cloud, the nubecula, after standing and cooling. Placed under the microscope this will show a few leucocytes and pavement epithelial cells derived from the mucous membrane of the bladder with a small amount of mucous from the genito-urinary tract. If strongly acid, the urine may deposit one standing a short time and cooling, a reddish precipitate of urates. This may be readily cleared up by boiling the urine.

Alkaline urine is usually cloudy when passed due to the presence of the phosphate and carbonate of lime. This may be cleared up by adding a small amount of acetic acid, enough to render the urine distinctly acid. The urine may be cloudy from the presence in the urine of blood, pus or bacteria, these urines are not cleared up either by boiling or by the addition of acid. Positive proof of the presence of these constituents is afforded by the microscope.

SPECIFIC GRAVITY.

The specific gravity of normal human urine varies from 1015 to 1025 with an average of about 1020. Great physical exertion and profuse perspiration may increase the specific gravity in perfectly healthy individuals to 1035. After excessive drinking the specific gravity of the urine may fall as low as 1002. The specific gravity of the urine is as a rule high in diabetes mellitus, in rheumatism, and most of the fevers. In chronic nephritis the specific gravity of the urine is usually decreased, and in general in any disease in which the quantity of urine is increased the specific gravity falls. The specific gravity is roughly a measure of the amount of solids in the urine. If the specific gravity of a specimen of urine above that of water (1000) be multiplied by the factor 2.33; the product will be approximately the number of grammes in 1000 cc. of the urine. As solutions of the different constituents of urine, of the same strength, have different specific gravities, it follows that this is only a rough working formula for the determination of the amount of total solids. The estimation of the specific gravity of urine is made by means of an instrument called the urinometer. This must be carefully constructed and graduated to register at a certain temperature. The cheaper urinometers are not graduated in this way, so that a good test of the accuracy of a urinometer is to notice whether it is graduated for a certain temperature. The urine is usually to be tested at a temperature of about 60 degrees F. and in no case must be tested while it is still sensibly warm. To avoid error the urine should be clear and should be poured into the urinometer carefully so that no bubbles are formed to interfere with the proper reading, Set the urinometer in a perfectly level position so that it does not touch the sides of the cylinder. Then place the eye on a level with the upper surface of the urine and read the specific gravity from the graduation opposite the lower border of the meniscus.

REACTION.

Normal urine has commonly a marked acid reaction to litmus, but after meals the urine may have a decided alkaline reaction. The acid reaction of the urine is probably due to the presence of acid sodium phosphate, produced by the reaction of the uric and sulphuric acids produced by metabolism on the basic sodium phosphate existing in the blood, hence it follows that the acidity of the urine is increased by exercise and the consumption of albuminous foods as also by the ingestion of mineral acids. The urine of a person living mainly on a vegetable diet is often distinctly alkaline.

The alkalinity of the urine may be due to the fixed alkalies, carbonates and phosphates of the alkali-metals or to volatile alkali ammonium carbonate. Ammonium carbonate is formed by the decomposition of urea and the determination of the form of alkali is made by moistening red litmus paper with the urine and

drying. If the alkalinity is due to fixed alkali the color is permanent but if due to ammonia the blue color fades as the paper dries.

The degree of acidity may be determined by diluting the urine (10 cc.) with distilled water (making up to about 50 cc.) adding a drop of phenol-phthalein and titrating with deci-normal caustic soda. When the urine is completely neutralised the fluid becomes of a faint pink tinge. The average amount of caustic soda solution required is about 3 cc. This may be stated as caustic soda or it may be expressed as oxalic acid. Each cubic centi-metre of soda solution represents an acidity equal to 0.63 Gm. of oxalic acid in 1000 cc. of the urine. If alkaline the alkalinity may be stated in the same way, substituting instead of the caustic soda solution, a decinormal-solution of sulphuric acid and titrating until the pink color of the phenol-phthalein disappears. The mixed secretion of the twenty-four hours should be decidedly acid.

ALBUMEN.

Under certain abnormal conditions any of the four proteids of the blood; serum-albumen, serum-globulin, fibrin and haemoglobin may appear in the urine. By far the most common one to appear is the serum-albumen. Egg-albumen may appear in the urine after ingestion of an excessive amount of egg-albumen in the food, as may certain of the products of the proteids such as proto, hetero, and deutero-albumose.

Albumen may appear in a number of marked conditions. The cause of albuminuria are classified as follows by Purdy:

1. Disease of the kidney substance.
2. Alterations in the blood.
3. Alterations in blood pressure.

DETECTION OF ALBUMEN.

All urine which is to be tested for albumen must be rendered faintly acid by the addition of acetic acid unless already so. Then filter the urine until perfectly clear.

1. Fill a small test tube nearly to the top with the suspected urine and boil. If albumen is present a cloud will form, even, if albumen is present in large amount, coagulating in large flakes, and, in cases where the amount is very large, coagulating completely. This may be due to an excess of the phosphates, which are easily differentiated by the addition of a few drops of DILUTE Nitric acid. If the cloudiness is due to the precipitation of the earthy phosphates, the precipitate will clear up immediately upon the addition of the acid while albumen will be thrown down in a more evident precipitate than before. This test while not very delicate, will detect albumen when present in anything more than a trace. To render the test more exact, two test tubes may

be taken and one boiled while the other is kept for comparison. A faint cloudiness is much easier to detect in this way, Or the urine may be boiled only at the upper part of the tube.

PURDY'S TEST.

To a small test tube nearly half filled with urine about 4 cc. of a 5% solution of potassium ferrocyanide is added and the fluid shaken, after which about one cubic centimetre of acetic acid is added. Any precipitate which may form is albumen and nothing else as no reaction occurs with mucin, peptones, urates, phosphates, alkaloids or resin acibs.

HELLER'S LAYER TEST.

A small amount of concentrated nitric acid placed in a small test tube, over this a layer of the filtered urine is placed by allowing it to flow slowly down the side of the test tube which, if properly done, will leave the urine and acid distinct. If albumen is present, a zone of coagulated albumen will form at the junction of the two fluids. This zone forms at once if there is a large amount of albumen present. When present in very small traces it may not become apparent for some little time so that in case of doubt, the test tube should be set aside for about five minutes before deciding that albumen is absent. If carefully applied this test is capable of detecting 1 part in 30,000, and is delicate enough for all practical purposes when carefully done.

TANRET'S REAGENT.

This reagent is prepared by the following formula:

Potassium Iodide 3.32 Gm.
Dissolve in 20 cc. of distilled water.
Mercuric Chloride 1.35 Gm.
Dissolve in 44 cc. of distilled water.
Mix and add 32 cc. of acetic acid.

This may be used as a layer test or by adding equal amounts of urine and the reagent. This reagent precipitates mucin, peptones and alkaloids but these substances are cleared up on boiling while albumen is not. It will detect 1 in 100,000 according to some authorities.

QUANTITATIVE ESTIMATION.

The amount of albumen may be estimated quantitatively by means of the Esbach albuminometer. This is a tube graduated for certain percentages of albumen by experiment. It is filled to the mark *U* with urine and the reagent then added to the mark *R*. The tube is then inverted gently a few times (about 12 times) without allowing any bubbles to become mixed with the albumen and set away in a level place for 24 hours. At the end

of this time the percentage of albumen is read off. Each division represents 1-10 per cent of albumen, or each division represents one gramme of albumen in 1000 cc. of urine. This instrument is only divided from 1 to 7 so that if the percent is above 7 the urine must be diluted one-half. If the quantity is below 1-10 per cent the instrument can not be used.

The most accurate way of estimating the amount of albumen is by precipitating the albumen by heat and acetic acid, and weighing the precipitate after drying for several hours at a temperature of 100 degrees *C.* The acid must be added a little at a time, boiling and filtering between the addition of the acid until the filtrate yields no precipitate by any of the albumen tests.

TITRATION METHOD.

Two solutions are required:

1. A standard solution of mercuric chloride 10 grammes in 1000 cc. of distilled water.
2. A 5% solution of potassium iodide.

Method: In a small flask place 5 cc. of the potassium iodide solution and a few drops of acetic acid. Add to this the mercuric chloride solution, drop by drop, until a distinct red tint appears. Place in the flask 10 cc. of the urine to be tested and again run in the solution of mercuric chloride until the red color which disappears in the presence of albumen again appears. Each cubic centimetre required for the second titration is equivalent to 0.0245 grammes of albumen. This test is based upon the fact that mercuric chloride in the presence of albumen and potassium iodide reacts first with the albumen and when all the albumen has been precipitated, reacts with the potassium iodide to form the red iodide of mercury.

GLOBULIN.

Globulin occurs in the urine quite frequently, usually with the presence of albumen at the same time. It is precipitated by the same reagents as albumen, and may be separated from that body by its insolubility in a saturated solution of Magnesium Sulphate.

DETECTION.

Twenty cubic centimetres of the urine is fully saturated with Magnesium Sulphate and filtered. The precipitate is washed with a saturated solution of Magnesium Suphate. Re-dissolve the precipitate in distilled water and on boiling the acidulated solution there will be a precipitate of the globulin. To estimate it quantitatively a larger quantity of the urine must be taken, and the second precipitate (that produced by boiling) weighed after

thorough drying. Or better still than weighing is the estimation of the amount of globulin by the amount of ammonia evolved and and titrated by the Kjeldahl method.

Albumen and globulin usually occur together and in variable proportions, but in general terms it may be stated that the greater the proportion of globulin the graver the prognosis.

SUGAR.

Sugar appears in the urine in two conditions, 1, as a temporary condition in which the excretion of sugar is due to the ingestion of an excessive amount of carbohydrates, to some nervous affection or to the ingestion of some drug such as chloroform or nitrite of amyl, etc., and 2, as the true diseased condition known as Diabetes Mellitus.

This disease is usually but not always attended by the passage of a large amount of urine (polyuria). In typical cases of this disease, the volume of urine excreted reaches from 3000 to 4000 cc. and may reach as high as 12,000 cc. per day. Typical diabetic urine is usually pale, but in mild cases not accompanied by polyuria the urine may be normal in color. The odor may resemble that of hay, and in advanced cases may give the odor of acetone or alcohol. The urine shows a marked tendency to froth when shaken and the froth is quite persistent. Its reaction is usually acid even after the administration of alkalies and may be extremely acid. The specific gravity of diabetic urine is high; averaging from 1035 to 1040. A case is on record in which the specific gravity was 1074 and the lowest density I have ever seen personally was 1024.

The quantity of sugar varies from a mere trace to as much as 600 grammes per day. The percentage of sugar also varies widely at different times in the day, being the least in the morning and greatest from three to four hours after a meal. In making a quantitative test therefore, the mixed twenty-four hour urine should be taken.

Dextrose is the most frequent form of sugar to occur in the urine, but lactose, maltose, sucrose or cane sugar, dextrin and inosite, other carbo-hydrates may occur in the urine.

TESTS.

One of the simplest and most reliable qualitative and quantitative tests for sugar is the fermentation test. This may be applied in two ways, the differential density method or by estimation of the amount of gas evolved.

The differential density method is employed as follows: The specific gravity of the specimen of urine is taken, then to about 100 cc in a bottle, add a small amount of yeast and allow to stand for about

twenty-four hours at the ordinary room temperature. At the end of this time the specific gravity is again taken and compared with the original specific gravity. Each degree lost corresponds to one grain of sugar to the fluid ounce, or the percentage of sugar may be obtained by multiplying the number of degrees lost by .23.

In Einhorn's saccharometer the amount of sugar is estimated by the amount of CO_2 evolved. If the amount of sugar is small, take ten cubic centimetres of the urine, add to it a small amount of yeast, free from sugar, shake together well and fill into the saccharometer until the long arm is entirely filled. Set away in some warm place for twenty-four hours, and then read off the percentage direct from the graduations on the tube. If the amount of sugar is large, two dilutions should be prepared, 1:5 and 1:10, and treated as above. If there is any doubt as to the purity of the yeast a blank test should be performed, using pure water, in this way testing for the presence of sugar in the yeast.

Trommer's Test: If urine or other liquid containing sugar be treated with sufficient of a solution of copper sulphate to render the liquid greenish, and a solution of caustic soda or caustic potash then added, a deep blue liquid will be formed if the urine contain a notable amount of glucose. On allowing the solution to stand for some hours in the cold, a yellow precipitate of cuprous hydroxide, or a reddish precipitate of cuprous oxide will form. This precipitate will take place immediately on boiling.

Bottger's Bismuth Test: The suspected urine is treated with an equal amount of caustic soda or caustic potash solution, a small amount (as much as will lie on the point of a penknife blade) of bismuth subnitrate and then boiled. If sugar is present, the bismuth will precipitated as the black metallic bismuth. In the presence of albumen this will give a black precipitate of the *sulphide* of bismuth, therefore albumen must be removed before this test is applied.

Picric Acid Test: Mix equal volumes of a cold saturated solution of picric acid and caustic soda solution and boil. Then add the urine to be tested and boil again for a minute or two. If sugar be present the solution will change color from a bright red to an intense brownish red, which becomes opaque in the presence of more than a trace of sugar. Unfortunately, creatinin gives a very similar color, normal urine giving a blood-red color which deepens on boiling.

Phenyl-hydrazine Test: 50 cc of the urine, free from albumen, is treated with two grammes of sodium acetate and one gramme of phenyl-hydrazine and boiled for half an hour. When cool, the phenyl-glucosazone which is formed, separates either as characteristic yellow needle-shaped crystals or as amorphous masses. In case it separates as amorphous masses, dissolve in hot alcohol, dilute with water, boil to expel the alcohol, and after cooling the crystalline deposit will form, which may be recognized under the microscope.

FEHLING'S SOLUTION.

Slightly acidulate and boil to precipitate albumen if present. Then add a few grains of sodium carbonate to render the urine distinctly alkaline and boil to precipitate the earthy phosphates. Filter.

FEHLING'S SOLUTION: (*a*) 34.64 grammes of pure sulphate of copper free from iron and moisture is dissolved in 500 cc of warm distilled water and filtered.

(*b*) 180 grammes of Rochelle salts are dissolved in about 300 cc hot water, filtered if necessary, and 70 grammes of good caustic soda or 100 grammes of caustic potash added to it and the solution made up to 500 cc.

Mix equal parts and the solution is ready for use. To avoid doubt from the presence of some other reducing substances it is best to treat the urine in the following manner:

To about 8 cc of the urine add 5 cc of the copper solution used in making up the test, after a preliminary boiling. Filter. Add to the filtrate 5 cc of the alkaline tartrate solution and boil for fifteen or twenty seconds.

If the solution has been kept for some time it may be tested by mixing equal parts (*a*) and (*b*) and adding an equal bulk of water and boiling. If there is no precipitate the solution is fit for use.

In ordinary cases it is sufficient to mix equal volumes of (*a*) and (*b*) and boil. While boiling add the urine drop by drop and if there is sugar present, it will be shown by a precipitate of the red cuprous oxide. Care must be taken that not more than an equal volume of urine is used to the original volume of the Fehling's solution.

QUANTITATIVE DETERMINATION.

BY FEHLING'S SOLUTION: A fairly accurate estimation of sugar may be made in the following manner; take 10 cc, of a freshly prepared Fehling's solution and dilute with 40 cc of distilled water and bring to boiling in a porcelain dish. Dilute the urine with from five to ten times its volume of distilled water and place in a graduated burette. Add the urine gradually to the copper solution, which is kept gently boiling until the blue color is entirely discharged. When this point has been reached stop the addition of the urine and note the exact amount of urine that has been used. This amount of urine contains .05 grammes of sugar.

INDICANURIA.

Indican occurs in the urine in the form of indoxyl-sulphate of potassium. It is a product of the action of putrefactive organisms in the intestine upon the proteids of the food, and in rare cases it is due to the presence in the body and absorption of decomposing pus. It is associated with impaired motility and paralysis of the intestine. The amount of indican formed and eliminated

is increased in sub- or an-acidity of the gastric juice, resulting doubtless from the fact that the food is not subjected to the action of a gastric juice, strongly acid enough to prevent the entrance of a large number of putrefactive organisms into the intestines. Clinically, then, it is increased in diarrhœas acute and chronic, in typhoid fever, in intestinal obstruction, in peritonitis, in gastritis acute and chronic, in gastric cancer, in cases where there is a large amount of pus retained in the body, and also in some cases of malignant disease.

Test: In a good sized test tube place 5 cc of urine and an equal amount of hydrochloric acid, two or three drops of a solution of sodium or calcium hypochlorite or a single drop of hydrogen peroxide and about 1.5 cc of chloroform and shake thoroughly, and finally allow to settle. If indican is present in excess, the chloroform is colored a deep blue, the intensity of the color being a fair index of the amount of indican in the urine. Unfortunately, there is no easy quantitative test for indican, and the amount therefore is best made comparative by always taking the same quantities of the reagents and estimating the amount by the intensity of the color produced.

RED INDIGO OR URRHODIN.

Red indigo or urrhodin appears under the same conditions as indican and appears to be a modification of the same coloring matter, probably an oxidation product.

Test: Treat the urine as given for ordinary indican as above, and instead of the blue color given by ordinary indican, the color will be red, the intensity being in proportion to the amount of the substance present. (2.) Boil the urine and while boiling add a few drops of nitric acid, boiling while the addition is going on. If indigo red is present, the urine will turn a deep red. On shaking the urine after this boiling, the foam will have a bluish-red color.

Care must be taken to exclude the presence of iodine. If present, this will impart a carmine tint to the chloroform, and even in the presence of indican will obscure the tint of the indican, or exactly simulate the tint of indigo red. The history of the patient will clear up this point, or the chloroformic extract may be evaporated to dryness and the residue may be tested for iodine by means of a weak starch solution.

CREATININ.

Creatinin is a normal constituent of urine, the amount present in twenty-four hours varying from 0.5 to 4.9 grammes, according to the amount of proteids eaten. The proportion is not decreased by fasting, but is said to be increased in typhus, typhoid and intermittent fevers, in pneumonia and tetanus.

It is diminished in convalescence from acute diseases, anæmia, chlorosis and phthisis.

ISOLATION.

Liebig's Method: The urine is exactly neutralized with milk of lime, and calcium chloride added as long as there is a precipitate of calcium phosphate. This is then filtered off and the filtrate, which should be neutral or faintly acid, is evaporated to a small bulk and the crystals of salt, etc., removed by filtration.

Thirty-two parts of the filtrate are then treated with one part of a very concentrated solution of zinc chloride and the whole allowed to stand for some days. The creatinin zinc chloride is washed with a little cold water and then with alcohol. It is then boiled with freshly precipitated lead hydroxide, the filtrate evaporated, and the residue digested with absolute alcohol, which dissolves the creatinin, leaving any creatine insoluble.

2) When a solution of picric acid is added to a solution containing creatinin and a drop of caustic soda solution is added to the mixture, which must have been well shaken previously, a a deep red color developes which is intensified by boiling. The intensity of this tint can be used as a rough quantitative test for creatinin. If creatinin is present in marked excess, the solution may become nearly black. This reaction will detect creatinin in solution when present in the proportion of 1 in 3000.

Sugar gives the same color with the solution, so this test is valueless for creatinin in the presence of sugar.

UROPHEN.

Urophen is considered by some authors to be only a mixture of the various coloring matters of the urine and the following test to be only a test for the total coloring matters of the urine.

Test: Four cubic centimetres of the urine are placed in a strong test tube and an equal amount of *strong* sulphuric acid is dropped into the urine and shaken.

In the presence of a normal amount of urophen the solution will acquire a deep brown color, which is transparent. In excess the color will be nearly black and not transparent. When subnormal the solution turns brown, often having a slight pink tinge.

Care must be taken in this experiment, for a great deal of heat is evolved and the tube is apt to break. To avoid risk it is better to do this test over the sink, using a test tube holder.

UROERYTHRIN.

Uroerythrin is the coloring matter of some of the pink urates. It occurs in excess in acute rheumatism and probably in some other acute diseases.

It may be extracted from the pink urate deposit by boiling alcohol. The residue after evaporation gives a green color when treated with caustic alkalies.

When in marked excess it may be detected by adding a drop of a solution of lead acetate to the urine in a clean test tube, and if in excess the precipitate which is produced will have a pink tinge, instead of the slightly yellowish tinge of normal urine.

UREA.

Urea is in warm blooded animals the main excretory product of nitrogenous metabolism. The amount varies quite widely, with the amount and kind of food, but is only slightly increased by exercise. By the action of the micro coccus ureae it is converted into ammonium carbonate, this being the cause of the alkalinity of urine after standing for some time in the air. It forms crystalline compounds with the stronger acids when they are added to a concentrated solution of urea. The nitrate is formed by evaporating urine to about one-fourth its volume and then adding nitric acid in excess, urea nitrate separates out, the fluid can be poured off and the urea nitrate strained out through muslin. The other salts are formed in a similar manner. If a pure product is desired the crystals may be purified by recrystallization from water, after filtration through animal charcoal. Urea may be separated from these compounds by the addition of barium carbonate in excess, and enough alcohol to form a pasty mass. Dry on the water bath, extract with alcohol, filter, evaporate the filtrate on a water bath, and set aside to crystallize. This is best decolorized by filtration through animal charcoal and recrystallization of the filtrate.

Estimation: Several methods have been devised for the estimation of urea. The simplest one is by the action of the alkaline hypobromites and measurement of the amount of nitrogen evolved. This is best done in the Doremus ureaometer. The following reagents are necessary:

1) A solution of 100 grammes of pure caustic soda in 250 cubic centimetres of distilled water.

2) Pure Bromide: For use the solution is made as follows: one cubic centimetetres of bromine is added to ten cubic centimetres of the soda solution and diluted with ten cubic centimetres of water. This mixture is placed in the ureaometer so that the long arm is completely filled. By means of the accompanying pipette, exactly one cubic centimetre of the urine is allowed to rise through the hypobromine solution in the long arm and the instrument set aside for fifteen minutes to allow the complete evolution of the nitrogen. At the end of this time, the amount of urea may be read off from the graduations on the side of the instrument. Each graduation corresponds to 0.001 gramme of urea in one cubic centimetre of the urine. The so-called percentage is found by moving the decimal point two places to the right. The amount in 1000 cubic centimetres is found by removing the decimal point three places to the right. The amount in twenty-four hours is found by multiplying the number of cubic centimetres by the amount of urea in one cubic centimetre. For various reasons the results found by the hypobromite test never correspond fully with the amount of nitrogen which theoretically should be found. It has been found from work on pure solutions of urea that from 88% to 94% of the urea is shown by the amount of nitrogen

evolved. The percentages are slightly higher when working on diabetic urine, and on urine to which glucose has been added.

The other tests are more complicated and not more accurate, and so are omitted, but may be found in any of the standard text books.

URIC ACID DETERMINATION.

25cc of urine is treated with an excess of ammonium chloride (about eight grammes being necessary) add ammonia until alkaline and allow to stand for about ten minutes, filter and wash with a cold saturated solution of ammonium chloride. Treat the precipitate with 20cc of a deci-normal solution of hydrochloric acid, boil for some minutes, cool, dilute to 200cc, add a few drops of methyl-orange and titrate with 1-10 normal caustic soda.

The difference between the amount of acid used and the amount of caustic soda represents the ammonia of the ammonium urate.

Each cc of the 1-10 soda solution shows the presence of 0.0168 gramme of uric acid.

If time is not essential, it is better to add the ammonium chloride and allow to stand for two hours, in which case the addition of ammonia is unnecessary.

The normal amount excreted is about .555 gramme, for the average man weighing 66 kilos.

It is much increased in rheumatism, fevers in general, and in that state called lithæmia, uric acid diathesis, etc., corresponding to the latent gout of English writers.

Total Nitrogen: The total amount of nitrogen is best estimated by the Kjeldahl method. This method is based upon the fact that nearly all nitrogenous bodies yield up their nitrogen in the form of ammonia when treated with concentrated sulphuric acid. Urea and uric acid readily undergo this change, and albumen after prolonged treatment, and passing through several intermediate stages finally is transformed into ammonia. 25cc of the urine is treated with 10cc of strong sulphuric acid in a porcelain basin, and boiled gently until reduced to about 10cc and the fumes of sulphuric acid are evolved. The residue is then placed in a pear-shaped digesting flask, the dish being rinsed with a few drops of water. Place the flask in an inclined position to prevent loss from spurting, and boil until frothing ceases. If the frothing is excessive, a small bit of paraffin may be added. After frothing has ceased, add about five grammes of potassium sulphate and boil until the solution is perfectly clear or only faintly yellowish. Allow the contents of the flask to cool and add about 20cc of water, cautiously. Prepare a concentrated solution of caustic soda and add slowly until the acid is nearly neutralized. This may now be treated in two ways. By the original Kjeldahl method, an excess of caustic soda is added to decompose the sulphate of ammonia and the whole mixture distilled, the ammonia collected and estimated by standard acid.

Or, the process may be modified by diluting the solution after nearly neutralizing it to 100cc and estimating the nitrogen by the hypobromite method.

CHLORIDES.

The chlorides in urine are best estimated by the following volumetric method. For this the following solutions are necessary:

1) Pure nitric acid Sp. Gr. 1,200.

2) Concentrated aqueous solution of ferric ammonium sulphate, free from *ous* salt and from chlorine.

3) Silver nitrate solution, 29.075 grammes to 1000 cc. 1 cc equals 0.01 NaCl.

4) Ammonium sulphocyanate, 25 cc of which equals 10 cc of the silver solution, or 1 cc equals 0.004 gramme of sodium chloride.

Method: 10cc of the urine, 50 cc of distilled water, 4 cc of the nitric acid, and 15 cc of the silver solution are placed in a 100 cc flask and well shaken and finally filled to the 100 cc mark. Filter through dry paper and take 80 cc for titration, which is equivalent to 8 cc of the urine. 5 cc of the ferric salt is now added, and the mixture titrated with the sulphocyanate solution till a red color appears, due to the formation of the sulphocyanate of iron. The quantity of sodium chloride in 1000 cc of the urine may be calculated from the following formula: x equals the amount of ammonium sulphocyanate solution used; then 37.5 minus x, multiplied by .004 equals the amount of sodium chloride in the amount of urine taken (8 cc), and from this the amount of the total chlorides is found by dividing by eight and multiplying by the number of cc passed in twenty-four hours.

The amount of chlorides excreted in the healthy adult varies from 10 to 16 grammes in the twenty-four hours, depending a great deal, however, on the diet of the individual. The excretion of the chlorides is diminished in all febrile states, and especially when attended with a serous exudation. A continued increase in the amount of chlorides excreted in the fevers, therefore, is an evidence of improvement. Their entire absence, therefore, in such a disease as pneumonia renders the prognosis grave.

PHOSPHATES.

The phosphates may be estimated approximately by adding to the urine (about ten cubic centimetres) about one-third its volume of magnesia mixture. There is immediately formed a white precipitate of ammonium, magnesium phosphate and of calcium phosphate. If normal in amount, there is a milky appearance; if deficient, there is only a slight turbidity, and if in excess, there is a heavy creamy precipitate. When accurate results are desired the best method of estimation is as follows:

Method: In an Erlemyer flask place 25 cc of the urine and add to it fifteen cubic centimetres of strong nitric acid (1.4). Bring to a boil and add, drop by drop, a saturated solution of permanganate of potash until there is a slight permanent precipitate of manganic oxide. Remove from flame and add about 1-30 gramme of white sugar to clear up the precipitate, boiling the solution again after the addition of the sugar. Remove from the

heat and add 13 cc of strong ammonia (.90). Insert a thermometer, bring to a temperature of 85°c, and add 50 cc of the molybdate of ammonia solution, wrap in a towel, cork and shake vigorously for five minutes.

At the end of five minutes filter on asbestos, wash with distilled water, and turn into the original flask.

Add 30 cc of the caustic soda solution and about 1 cc of phenol phthalein solution and finally titrate back with nitric acid solution. Each cubic centimetre of the alkali in excess of the acid used represents 0.001 grammes of P_2O_5.

Solutions Needed: 1) Nitric acid (1.4).

2) Ammonia (.90).

3) Caustic soda solution, made up by taking 323.7 cc of a normal caustic soda solution and making up to 1000 cc.

4) Nitric acid equivalent to the solution of caustic soda.

5) Molybdate of ammonia solution, made by taking 90 grammes of ammonium molybdate, and dissolving in slightly less than 1000 cc of distilled water, allow to settle, decant, and dissolve the residue in ammonia, add to the solution and finally make up to 1000 cc. Each cc will precipitate .003 grammes of P_2O_5.

SULPHATES.

100 cc of the urine is boiled with 5 cc of strong hydrochloric acid (free from sulphates) and 5 cc of a 10% solution of barium chloride. The boiling must be kept up for at least an hour. At the end of this time the urine is filtered through a fine, close filter and washed with boiling distilled water. Ignite in a weighed porcelain crucible to a white ash. After cooling, weigh. The difference in weight will represent the weight of barium sulphate formed from the sulphates of the urine. The amount of sulphur trioxide in 1000 cc may be found by multiplying the weight of barium sulphate found by the factor 3.4333.

VOLUMETRIC METHOD.

Solutions Required: Solution of barium chloride (crystallized) 30.5 grammes in 1000 cc of water.

Solution of potassium sulphate 20%.

Pure hydrochloric acid.

One hundred cubic centimetres of the urine is treated with 5 cc of hydrochloric acid and brought to the boiling point in a flask. The barium solution is then dropped into the flask as long as there is a precipitate formed, the mixture being heated before each addition of the barium solution. After adding about 5 or 6 cc of the solution allow it to settle and draw off a few drops of the clear solution above and test with a few drops of the barium solution. If any precipitate occurs, return the whole to the flask and add a small amount of the barium solution as before, allow to settle and test as before. The end of the reaction is reached

when the drop of clear fluid removed gives no precipitate with the barium solution and just gives a faint trace of cloudiness on the addition of a drop of the potassium sulphate solution.

Each cc of the barium solution required represents 0.01 gramme of sulphuric acid.

ESTIMATION OF PREFORMED SULPHATES.

Method: 100 cc of clear filtered urine is mixed with an equal volume of an alkaline barium chloride solution (two volumes of barium hydrate solution and one volume of a barium chloride solution, each saturated at the ordinary temperature) strongly acidified with acetic acid and stirred thoroughly for several minutes. This is then filtered, the precipitate washed with distilled water incinerated to a white ash and weighed. This will give the amount of preformed sulphates, the sulphuric acid in combination with sodium and potassium. The difference between the total sulphates and the preformed sulphates will give the amount of ethereal sulphates, the sulphuric acid in combination with phenol, indoxyl and skatoxyl. These are products of intestinal putrefaction and are increased in diseases with increased intestinal putrefaction. The normal relation between preformed and ethereal sulphates is 10-1, but the ratio may fall immensely in such troubles as intestinal obstruction.

BILE PIGMENTS.

Gmelin's Method: Fuming nitric acid is placed at the bottom of a test tube and the urine allowed to flow gently down the sides of the test tube and form a layer above the acid. If the bile pigments are present there will be a green ring at the line of contact and below it in order, rings of violet, red and yellow will appear.

Rosenbach's Test: The urine is filtered and the filter paper, which will be stained yellow, is touched with a drop of fuming nitric acid. If bile pigments be present, the rings will be formed as in Gmelin's test.

Rosin's Test: On the surface of the urine in a test tube, dilute iodine solution is floated. If bile pigments be present, a grass-green zone will be formed at the line of junction of the two fluids.

BILE ACIDS.

Bile acids in small traces occur even in normal urine. They are increased in any condition in which there is a deficient elimination of bile, such as early cirrhosis, hepatic congestion, malarial poisoning, hepatic tumors, cancer and amyloid disease.

Oliver's Peptone Test: Peptone, grammes 30.
Salicylic acid, grammes 4.
Acetic acid, half ounce.
Distilled water to make eight ounces.
Dissolve all the ingredients and filter until perfectly clear.
Prepare the urine by rendering it acid and diluting to a specific gravity of 1008 and filter until perfectly clear.

To 3 cc of the test solution in a test tube, add 1 cc of the urine. If bile acids be present in excess, a distinct milkiness will at once appear. If 3 cc of the urine are required to produce the milkiness, bile acids are not present in excess.

ACETONURIA.

Acetone occurs in the urine in a variety of conditions. It is stated by some authors to occur in very minute quantities even in normal urine, but only about 0.01 grammes per day.

It occurs in many of the fevers and is then called febrile acetonuria. It is increased in most cases of diabetes in the later stages being often found in great excess. It occurs also in inanition, carcinoma and in psychoses.

Tests: Small amounts of tincture of iodine and a solution of caustic potash are added to the urine and it is allowed to stand for a short time, when, if acetone is present in the proportion of one in 5000 crystals of iodoform will be precipitated and may be recognized by their form under the microscope.

Chautard Test: Distill 200cc of the urine at a gentle heat and test the first 15cc of the distillate with sulpho-rosanalinic acid. If acetone is present, a fine reddish-violet coloration appears, the intensity of which varies with the amount. It is stated that less than 0.01 per cent. can be detected in this manner.

Sulpho-rosanalinic acid, the reagent used in the above test, can be prepared by dissolving 0.25 gramme fuchsine in 500 cc of water, then passing a current of sulphur di-oxide through it until the yellow color disappears. As it is very sensitive to oxidising agents it must be kept in a well stoppered bottle.

DIACETURIA.

Diaceturia never occurs in health and is shown by the presence of diacetic acid in the urine. It occurs (with a simultaneous abundance of acetone) in diabetes, especially when severe and about to go into coma, and in febrile affections, especially in children. It is supposed to be the cause of the convulsions which occur so frequently in the course of febrile affections in children.

Tests: Add some solution of ferric chloride, a precipitate may form of the phosphates and if so must be filtered off. More iron chloride must be added and if diacetic acid is present the urine becomes a Bordeaux-red. Then repeat with urine that has been

boiled. Then mix a portion of the urine with sulphuric acid, extract with ether, and repeat the chloride of iron test with the ethereal extract.

Diacetic acid is present, if in the presence of the chloride of iron reaction the following reactions occur:

1) The urine after boiling shows little or no reaction with the chloride of iron test.

2) The ethereal extract shows a chloride of iron test fading in twenty-four hours or less.

3) When acetone is also present.

AMMONIA.

Of almost equal value is the determination of the amount of ammonia in the urine. Ammonia seems to be formed by nature in an attempt to neutralize the acids which are formed in the system antecedent to the acetone and diacetic acid and which are supposed to be the cause of the coma and other nervous disturbances.

DETERMINATION: Take 10 cc of urine and place in a flask and exactly neutralized with a deci-normal caustic soda solution, using Rosolic solution as an indicator. 10 cc of a deci-normal sulphuric acid are then added and boiled to expel carbon dioxide. Deci-normal caustic soda is then added until the red color is just restored. The number of cc of deci-normal sulphuric acid less the number of cc of caustic soda, the result multiplied by 0.0048 gives the number of grammes of ammonium carbonate in 10 cc of the urine.

In diabetes, if the patient is excreting more than 1.1 gramme per day he is suffering from a severe attack of the disease. If he is excreting more than this, two, four, six or more grammes of ammonia per day, he is in danger of passing into diabetic coma. Care must be taken in this test that the urine is fresh, if allowed to stand for some time the amount of ammonia is increased by the fermentation and decomposition of the urea. Care must also be taken that the urine is exactly neutralized before the sulphuric acid is added, as the normal acid reaction of the urine will neutralize some of the alkali if not exact.

BLOOD AND BLOOD PIGMENTS.

Tests: HELLER'S TEST: A small portion of the urine in a test tube is made distinctly alkaline with caustic potash and boiled. The phosphates are precipitated and carry down with them the coloring matter of the blood, giving the precipitate a brown or reddish-yellow color. Should the urine be poor in phosphates the suspected urine should be mixed with an equal amount of urine containing a normal amount of phosphates and the test applied.

GUAIAC TEST: For use equal parts of *fresh* tincture of guaiacum and *old* oil of turpentine are mixed in a test tube and

the urine floated carefully over it. At the line of junction, in addition to the deposit of resin, an indigo-blue ring will develop, and on shaking will give to the whole fluid a non-transparent blue color, fading in a few minutes.

HAEMIN TEST: A drop of the urine is evaporated on a glass slide, with a crystal of common salt. The deposit is then moistened with a drop of glacial acetic acid and boiled. When cool examine under the 1/6 lens, and if blood or haemoglobin be present the characteristic rhombic brownish crystals of haematin hydrochloride will be seen.

Haematuria can be differentiated from haemoglobinuria by microscopical examination. In the former we find the above color reactions, and in addition the presence of a number of red corpuscles, while the latter shows the chemical reactions for blood, while microscopically few or no red corpuscles can be seen.

DIAZO REACTION.

It has been found that in most cases of typhoid fever, and in some other diseases such as measles and tuberculosis, the urine contains some undetermined compound which gives a peculiar reaction with diazosulphobensol.

To secure fresh diazosulphobenzol Ehrlich uses two solutions. No. 1 a ½% solution of sodium nitrite, and No. 2 containing two grammes of sulphanilic acid and 50 cc of hydrochloric acid in 1000 cc of water.

Test: Forty parts of No. 2; one part of number one; forty-one parts of urine are thoroughly mixed in a large test tube. Strong ammonia is floated on this mixture.

If the diazo reaction is present, a carmine red ring will form at the junction of the two fluids, after shaking the foam will have a characteristic salmon-pink color, and after standing for twenty-four hours there will be a greenish precipitate.

The urine must be perfectly fresh for success with this reagent.

MICROSCOPIC EXAMINATION.

By sediment in the urine we refer to any deposit from the urine which is visible to the naked eye, or can be detected by microscopic examination.

The sediment is best obtained by means of the centrifuge, by the use of which we get the unchanged sediment of the urine, unaltered by standing and fermentative processes. As these instruments are expensive, most physicians will content themselves with a cheaper apparatus. Very satisfactory examinations may be made by allowing the urine to stand for about two hours in a conical glass, then withdrawing the sediment with a pipette, placing a single drop on a clean glass slide and covering with a

clean cover-slip. This may be examined with an objective magnifying about 400 diameters (about 1-5 or 1-6 objective.)

The sediments occurring in the urine may be divided into two classes, organic and inorganic.

ORGANIC SEDIMENTS.

Mucous occurs (1) in small granules, visible to the naked eye, which, under the microscope prove to be aggregations of pus corpuscles, held together by a small amount of transparent mucous.

2) In the form of microscopic threads one to two centimetres in length, which under the microscope may consist of a closely packed thread of pus corpuscles held together by mucous, or may consist almost entirely of mucous with a few pus corpuscles attached to them. These are the so-called "mucous threads" of gonorrhoea, and are often found to contain the gonococci in cases of chronic gonorrhoea in which the discharge may have ceased entirely.

3) Mucous also occurs in the form of microscopic threads, "cylindroids" which resemble very much some forms of renal casts. Usually they may be easily distinguished from casts by their irregular outline, being thin in one place, quite thick in another, by the appearance of mucouslike striation, and by their considerable length.

Red Blood Corpuscles: Red blood corpuscles in the urine may be due to hemorrhage anywhere in the urinary tract. When from the urethra the blood is passed with the first portion of the urine, is often bright red and frequently is coagulated in a kind of cast of the urethra.

Haematuria, the blood coming from the bladder, is characterized by the variable amount of blood, and by the fact that the blood is not intimately mixed with the urine, often being present in distinct clots.

Vesical haemorrhage may be due to severe cystitis, tumors, especially in villous growths, stone and in a few rare cases due to the presence of various parasites, such as a variety of *cercomonas*.

Haemorrhage from the pelvis of the kidneys resembles the haemorrhage from the bladder, and may be accompanied by the passage of coagula molded to the form of the ureter. The most common causes of haemorrhage from the pelvis of the kidney are the presence of stones in the pelvis and tumors.

True renal haemorrhage occurs in acute and chronic haemorrhagic nephritis, in infarction, marked renal congestion (usually due to irritating drugs) in tumors and injuries of the kidneys. It occurs also without kidney lesions in malaria, scorbutus and in the haemorrhagic diathesis. In true renal haemorrhage the blood is intimately mixed with the urine, giving the urine a smoky or brownish hue. The corpuscles under the microscope may retain their normal shape and color, or they may have lost their color and appear as clear colorless rings. The corpuscles may be

formed into the blood-casts (see below) and are then characteristic of renal haemorrhage.

Pus Cells: Pus cells occur in the urine whenever there is suppuration going on in any part of the urinary tract, and in smaller numbers, in acute and chronic nephritis. They may be due also to the rupture of a collection of pus from outside into the tract. They appear as small round granular bodies, with one or more nuclei, which may be rendered more distinct by the addition of a little acetic acid, or by treatment with staining agents such as methylene blue. A few pus cells may occur in the urine in a normal individual, and it is only when present in quantity that they become pathological.

Epithelial Cells: Epithelial cells may be derived from any part of the urinary tract and occur in any inflammation of the mucous membrane lining this tract. They vary much in size and shape, and no definite conclusion can be drawn from the size and shape with regard to their source. In general, those from the bladder are large, thin, flat, polygonal cells with a small clear nucleus. Those from the deeper layers of the bladder have the appearance of columnar cells, or caudate, smaller in size with a larger nucleus. Epithelium from the renal tubules are small, not much larger than a lencocyte, are irregular in outline but usually rounded, and contain a large distinct nucleus. It is impossible to say that any single cell is from a definite place in the urinary tract, but when numerous cells are seen, having the general characteristics of one of the classes as given above, a fairly accurate opinion can be arrived at by a careful observer. In women, care must be taken to prevent contamination of the urine by any vaginal discharge. This is best avoided by drawing the specimen with a catheter, or in cases where this is impossible, by giving the patient a vaginal douche before the specimen is passed.

Tumor Cells: Bits of tumors are sometimes passed in the urine, but rarely in such a form that they are available for diagnosis. In the cases in which bits of tumors are passed they should be hardened as any tissue for microscopic examination, and after hardening, cut with the microtome. The diagnosis of these tumors belongs to the pathologist.

Spermatozoa: After every discharge of semen these are to be found in the urine and are easily recognized. They are found after epileptic attacks and occasionally in severe diseases of all kinds.

Casts: These are the most important of the formed elements in urine, and always occur with albuminuria. They vary much in size, form and composition in the different varieties of renal disease.

The following varieties may be described:

Hyaline Casts: These vary much in size, sometimes narrower than the diameter of a white corpuscle, again four or five times as wide, and their length may be as great as one millimetre. They are colorless and transparent, and may be easily overlooked by a careless observer. Their ends may be rounded or they may look as though broken off. In many cases various urinary

deposits and other bodies, such as red and white corpuscles, may adhere to them and render them difficult of recognition. These hyaline casts, with granular material adherent to them, are called granular casts.

Blood Casts: These are collections of red blood corpuscles, held together by coagulation. These only occur in renal haematuria.

Epithelial Casts are either hyaline casts, with epithelial cells adherent to them, or they are true casts of the tubules, a portion of the epithelial lining of the tubule having been exfoliated and washed down in the urine without disintegrating. They are most frequently found in acute nephritis, but may occur in any form, least frequently in the small granular kidney (chronic interstitial nephritis).

Parasites: Animal parasites in this country are infrequent, usually when found in the urine being due to contamination from the vagina. Oxyuris vermicularis and trichomonas vaginalis occur in this way. In rare cases, a species of Cercomonas, resembling that found in the vagina and the one occurring in the intestine, is found in the urine, and in these cases there is usually a severe form of cystitis, with occasional haematuria, the urine retaining its normal acidity, or the acidity may be increased.

Vegetable Parasites: Many different forms of bacteria may be found in the urine under diseased conditions. In the healthy individual the urine as drawn from the bladder will be found aseptic.

After standing even a short time the micrococeus ureæ develops and is the factor which transforms the urea into ammonium carbonate and gives the urine an alkaline reaction after standing.

In cystitis we find a number of bacteria, the commoner ones being colon bacillus, streptococcus and staphylococcus pyogenes aureus and the gonococcus. In a few cases the typhoid bacillus may be the cause of cystitis. The tubercle bacillus is not infrequently found as the cause of a chronic ulcerative cystitis and should be sought for in the sediment. The culture and differentiation of these bacilli belongs to the bacteriologist.

Many other bacteria may occur in the urine in disease, often passing down from the kidneys in infectious diseases.

Yeast cells occur in diabetic urine, usually in abundance and multiply rapidly, they occur occasionally in urine not containing sugar, but in these cases do not increase.

INORGANIC SEDIMENTS.

The character of the crystalline deposit in urine varies with the reaction of the urine.

In acid urine the usual deposits are of potassium, calcium and sodium urate (amorphous), uric acid and calcium oxalate.

From alkaline or neutral urine the deposits are usually the ammonio-magnesium phosphate (triple phosphate), phosphate and carbonate of lime, urate of ammonia, and rarely uric acid.

Uric Acid: As stated before this may be present in acid, neutral or rarely in alkaline urine. It may often be detected by the naked eye as reddish grains clinging to the side of the reagent glass. Microscopically it presents numerous forms, "whetstone"

shape, barrel shape, and crosses and more frequently still amorphous masses of a reddish-yellow color or in superimposed plates of the same color. Uric acid precipitated from the urine always has this color, due to the coloring matters of the urine carried down with it (probably in most cases uroerythrin), while chemically pure uric acid is colorless.

The separation of uric acid crystals does not indicate an excess of uric acid, as it may be thrown down in any urine after standing a short time.

Urates of sodium, potassium and calcium occur as the reddish "brickdust" precipitate which separates on cooling. They are immediately dissolved on warming, and under the microscope are seen as fine granules.

Oxalate of Calcium: A few crystals of this may separate from any urine after standing a short time. In some cases they are very abundant, making up nearly the whole of the sediment. Their presence does not indicate necessarily an excess of oxalic acid, which can only be proven by chemical tests. They are in excess after eating certain vegetables and fruits, apples, pears, cauliflower, rhubarb and various sorrels, and in diabetes mellitus, hypochondria and in some cases of chorea.

Its most common form in the urine is the small nearly square envelope form, more rarely it occurs in hour-glass or dumb-bell forms. They are insoluble in acetic acid and colorless, and are thus distinguished from triple phosphates.

Triple Phosphates: These are the commonest crystals seen in alkaline urine and present themselves in numerous shapes. They occur in the coffin lid or whetstone form, as envelopes nearly square, as feathery crystals, and as thin plates. Some of the forms resemble calcium oxalate crystals, but may be differentiated from them by their solubility in acetic acid.

Ammonium Urate: Urate of ammonia is present in all urine which is alkaline from fermentation. It is precipitated in reddish crystals, some of them being simply round spherules, others as reddish spheres with sharp spines projecting from them, the so-called "hedge-hog" crystals.

Phosphate of Calcium is found as thin colorless plates in simple alkaline urine, and is destroyed by fermentation.

Carbonate of Calcium occurs as colorless granules, often associated in pairs. It is found in simple alkaline urine and in urine alkaline from fermentation.

Cystin is a rare sediment, occurring in thin, colorless polygonal plates, and may be a constituent of urinary calculi.

Leucin and Tyrosin: These rare substances usually occur together, and may be present in the sediment, or may appear only when the urine has been evaporated to a syrupy consistency. Leucin appears as faintly shining spheres, and if large may show concentric rings and radiating lines. Tyrosin occurs as fine needles, usually collected into bundles.

They are found in acute yellow atrophy of the liver, in acute phosphorus poisoning, occasionally in typhoid fever, smallpox and pernicious anæmia.

BLOOD.

In examining the blood, the main points to be noticed are the amount of haemoglobin, the number, form, size and variations in color of the red corpuscles; the number, classes and proportions of each variety of the lencocytes, and the occurrence of parasites.

HAEMOGLOBIN: The amount of haemoglobin may be estimated by the appearance of the mucous membranes, by the appearance of a drop of the blood, or by some form of haemoglobinometer. A deficiency in the amount of haemoglobin, if very marked, may be easily detected by the pallor of the mucous membranes, and by the pale, watery appearance of the blood when drawn.

The most accurate clinical test is by the use of the haemoglobinometer. Several forms of this instrument are in the market, the best probably being the instrument devised by Fleischl. A certain small quantity of blood, obtained by pricking the end of the patient's finger or the lobe of the ear, is measured by filling a capillary tube. The blood is washed into a chamber with distilled water and the chamber exactly filled with the water. The other division of the chamber is filled with water, the chamber placed on the stand of the instrument, a long hollow cylinder painted black on the inside. is placed over it to exclude all light except that from the mirror below, and the color compared with that of a colored glass wedge which fits in the stage. This glass wedge is moved by means of a thumbscrew until the tints are exactly the same, when the percentage of haemoglobin may be read off from the graduated scale. Artificial light must be used in this determination, so the test must be conducted in a darkened room. The amount of haemoglobin in general corresponds closely to the number of red corpuscles, in only two blood diseases is there any great disparity. In chlorosis the number of red corpuscles may be normal or only slightly decreased, while the haemoglobin registers very low, falling as low as 30 per cent. On the other hand in pernicious anæmia the number of red corpuscles falls very low, even to less than 1,000,000 to the mm^3 and while the percentage of haemoglobin falls very low it never falls as low in proportion as the red corpuscles.

RED CORPUSCLES: The red corpuscles are best counted by the Thoma-Zeiss apparatus. This consists of a pipette graduated accurately, and a glass plate, divided by means of fine lines into minute squares. This small glass plate is placed on a glass slide and surrounded by a glass plate, 1-10 millimetre thicker than the graduated plate. This leaves a well 1-10 of a millimetre in depth, the bottom of which is formed by the graduated plate. This is subdivided by double lines into sixteen large squares, and each of these is again sub-divided into sixteen smaller squares. These

smaller squares measure 1-20 of a millimetre on each side, thus giving a chamber over each square 1-20x1-20x1-10, having a capacity of 1-4000 mm³.

The finger or lobe of the ear is cleaned thoroughly and sharply pricked with a clean needle. A drop of blood is allowed to exude, avoiding pressure if possible. Take the pipette and draw the blood up to the graduation 0.5 or 1.0 and then draw in the diluting fluid to 101. Shake well to thoroughly mix the blood, blow out the drop of salt solution in the capillary tube, then blow a small drop of the blood on the centre of the glass plate, cover with the cover-glass (avoiding bubbles of air) and after a short interval to allow the corpuscles to become quiet, examine under the 1-5 or 1-6 inch objective.

Care must be taken that the drop of blood is not too large and that it does not flow off from the small glass circle upon the large outer plate.

After focusing the microscope, commence counting on the upper left-hand square of the field, counting those that touch the upper and left-hand lines and all those that lie free in the square, but not those that touch the lower and right-hand lines bounding the square. This is to avoid counting any corpuscle twice. After counting the sixteen squares seen under the field, the slide is to be moved and another set of sixteen counted. Three different drops are to be counted in this way and the average of the six counts taken for the calculations to secure accuracy.

The calculation is as follows: Divide the average obtained, by sixteen to find the average number in one of the smaller squares. If the blood was drawn up to the mark 0.5 multiply by 200, for in this case the blood was diluted 200 times; if to the mark 1.0 then by 100 for the dilution was then 1:100 and finally multiply by 4000; for the small square, the unit of capacity was 1-4000 of a mm³. This gives you the number of red corpuscles in a mm³. In health this averages in the male 5,000,000, in the female 4,500,000. As a diluting medium it is best to use a normal salt solution, six gramme of sodium chloride to 1000 cc of water.

White Corpuscles: The same glass slip is used in counting the white corpuscles as was used for the red corpuscles. A special pipette giving a dilution of 1:10 or 1:20 is used for mixing the blood. The blood is diluted with a 0.3% solution of acetic acid, or by a staining solution which I have found more satisfactory. This is made up by adding to the 0.3% solution of acetic acid, enough methylene blue to make a bright blue. After standing a short time the nuclei of the white corpuscles will be stained a distinct blue, while the red corpuscles are completely decolorized by the acid. It is best in counting the leucocytes, to count all the corpuscles in the sixteen *large* squares, and repeat this on five successive drops, finally making the calculations from the average of these. The calculations are as for the red corpuscles, allowing for the different number of squares and different dilution.

Differential Staining: In the normal blood five different kinds of colorless corpuscles can be distinguished.

1) Small leucocyte or lymphocyte.
2) Large leucocyte or lymphocyte.
3) Transitional forms.
4) Polynuclear, neutrophilic leucocyte.
5) Eosinophile.

In some diseased conditions a sixth corpuscle makes its appearance, the myelocyte.

To prepare specimens of blood for staining the cover-glasses are carefully cleaned in dilute nitric acid and alcohol and carefully dried. The finger is cleaned thoroughly, dried, then rubbed vigorously to stimulate the circulation, a small puncture is then made with a clean needle, and the blood allowed to exude. One of the cover-glasses is placed on the drop, then placed on another glass to spread the drop thinly and evenly, and finally they are drawn apart, care being taken to draw them apart in the same plane.

If the examination is for the demonstration of micro-organisms all the minutiae of disinfection must be gone through with.

These thin specimens soon dry in the air, if properly made, so rapidly that the red corpuscles do not become crenated or misshapen. These specimens may be hardened in several ways. They may be heated to a temperature of 120°c for an hour, or hardened in a mixture of equal parts of absolute alcohol and ether for an hour, or in bichloride 1:1000 or 2% solution of formaline for the same length. The alcohol and ether hardening has on the whole given me the best results, the heat method coming next in efficiency.

After hardening in this manner they may be preserved indefinitely if preserved from dust and moisture. When ready for examination they may be stained with any of the analine stains or with haemotoxylon and eosin. If eosin is used, it must be an alcoholic solution of about 70% alcohol.

For routine work, it is better to use some stain made up on the plan of Ehrlich's triple stain. The one in use in the laboratory is made up from the following formula: Make saturated aqueous solutions of acid fuchsine, methyl green crystallized and orange G. extra.

Take of the saturated solution of orange G. 55 cc, of the solution of acid fuchsine 50 cc and add to them 100 cc of distilled water and 50 cc of absolute alcohol and shake.

Take of the saturated solution of methyl-green 65 cc, add to it 50 cc of distilled water and 12 cc of absolute alcohol, shake well and add to the first mixture drop by drop, shaking between the addition of each drop.

With this stain the nuclei of the leucocytes are stained green, the nuclei of the nucleated red corpuscles if any be present, an intense green nearly black, the neutrophilic granules a purple, the eosinophilic granules a bright red.

This stain must stand for about three weeks before it is fit for use, and then is used by simply putting a few drops on one of the covers prepared as above, allowing it to remain about four minutes, wash with water, dry quickly in filter paper, and mount in balsam. Examine under the 1-12 oil-immersion objective with the Abbe condenser.

The small mononuclear leucocyte with this stain presents a single green nucleus, surrounded by a very narrow rim of pink protoplasm, not larger than the red corpuscles.

The large mononuclear leucocyte presents a single greenish nucleus about the size of the preceding, round, surrounded by a comparatively wide margin of pink protoplasm, not granular. This cell is larger than the red corpuscle, its main distinguishing feature from the small leucocyte.

The transitional leucocyte is about the same size and appearance as the large mononuclear leucocyte, the nucleus, however, showing irregularities and the protoplasm occasionally shows evidence of beginning granule formation.

The polynuclear neutrophile presents from two to six distinctly stained green nuclei, a wide rim of protoplasm with a large number of small purplish granules. It is much larger than any of the preceding, being from 12 to 14 micro-millimetres in diameter on the average.

The Eosinophile resembles the preceding, but its granules with the stain mentioned or with eosin and haemotoxylon are large, distinct, red, and often overlap the nuclei.

The myelocyte is stated to be diagnostic of leucocythæmia. It is a large mononuclear, neutrophile, the nucleus large, not staining very distinctly, the protoplasm presenting the small purple granules, resembling those of the polynuclear neutrophile.

The normal red corpuscle with this stain, is a bright buff tint, in some cases of disease, showing much lighter than normal, this condition being found in many of the acute infectious diseases.

The nucleated red corpuscle appears a distinct buff color with a single nucleus, staining intensely and appearing almost black. This occurs normally in the blood of infants, and is found after severe haemorrhage in leucocythæmia, pernicious anæmia, and occasionally in any severe anæmia.

The proportion of each of these forms of white corpuscles varies, but the normal blood will show from 60 to 70% of polynuclear neutrophiles, from ½ to 2% of eosinophiles, from 6 to 12% of large mononuclear leucocytes, from 1 to 3% of transitional leucocytes, and from 15 to 25% of the small mononuclears. The proportion of white to red corpuscles in health varies from 1:400 to 1:700. A temporary increase or an increase not exceeding 1:20 is usually called leucocytosis. Leucocythæmia, on the other hand, is characterized by an enormous increase in the white corpuscles, which may reach the proportion of three white to two red corpuscles, and by the presence of the large mononuclear neutrophile cells

mentioned above. Under treatment the proportion of lencocytes may become nearly normal, but the myelocytes are persistent and characteristic of the disease.

Leucocytosis occurs in the acute infectious diseases, with the single exceptions of typhoid fever and acute miliary tuberculosis, it occurs with suppuration in any part of the body, it occurs in the second and third stages of tuberculosis when the disease has has gone on to cavity formation. A physiological lencocytosis also occurs after meals. The normal number of lencocytes varies from 4000 to 6000, and in these diseases may be increased to 30,000 or 40,000.

Not much can as yet be said of the diagnostic significance of variations in the proportions of the different varieties.

The blood may be examined for parasites, either fresh or in the stained specimens. Parasites of the blood may be either animal or vegetable. In this country almost the only animal parasite is the plasmodium or Haemotozoön malariae. This is always found in malarial fever of any type, and is the only absolute diagnostic point. Many different forms of this parasite are known, but may be grouped into a few general classes.

1) Colorless bodies embedded in red corpuscles, about one-fourth the size of the corpuscle, and possessing amoeboid movement.

2) Bodies resembling the preceding form, but containing a considerable amount of small granular brownish pigment.

3) Large clear bodies, larger than a red corpuscle, containing more or less of the brownish pigment granules, usually arranged about the periphery of the body.

4) Small bodies about one-quarter the size of a red corpuscle, lying free between the cells, and possessing active movement. These are the so-called spores.

5) Small round motile bodies larger than the preceding having one or more cilia; called flagellate organisms.

6) Crescentic shaped bodies, with or without a small amount of pigment, embedded in the thickest portion of the crescent near the center.

7) Bodies composed of small irregularly rounded particles grouped around a central mass of pigment.

These parasites are best studied in the fresh specimens without staining, using for the purpose the 1-12 inch oil-immersion objective. If thought necessary, the specimens may be prepared as described above, and stained with aqueous methyline-blue solution or with other of the aniline dyes.

The vegetable parasites include any of the pathogenic bacteria, and require special bacteriological technique for their detection, hence the main part of this subject must be given by the bacteriologist. In general, it may be said that the blood is drawn and the specimen prepared under strict aseptic precautions, the hardened specimens stained with the aniline dyes and examined under the 1-12 objective.

EXAMINATION OF SPUTUM.

By expectoration we mean any substance thrown out of the air passages by the act of coughing or hawking. Microscopically we can distinguish the following varieties of expectoration:

1) Mucous sputum is either glassy and transparent or grayish white, usually quite tough. It occurs in acute bronchitis, and often has its source in the oro- or naso-pharynx.

2) Muco-purulent sputum consists of a mixture of mucous and pus in varying proportions. Pus is recognized by its yellowish green color and its opacity.

3) Purulent sputum. Pure pus is not a common form of sputum. It occurs from the sudden rupture of an abcess or empyema and occasionally from the sudden emptying of a cavity. It is recognized by its color, opacity and lack of toughness and stickiness.

4) Serous sputum is the diagnostic expectoration of oedema of the lungs. It is very fluid, but not so much so as serum, as it always contains more or less mucous. It may be light gray and transparent or may contain enough blood to give it the appearance of "meat-washings."

5) Bloody sputum. Any of the preceding forms of sputum may be tinged with blood or may contain small streaks of it. In pneumonia the characteristic sputum is a tough glassy mucous tinged with the coloring matters of the blood, which may render it bright-red, yellowish-red, rusty or even greenish. Sometimes blood may be the chief constituent of the sputum and in these cases constitutes haemoptysis.

The odor of sputum is ordinarily stale, but in chronic bronchitis with bronchiectases, in large cavities with decomposing secretion as in the last stages of phthisis and in gangrene of the lung the sputum may be very offensive.

Foreign Substances: By the unaided eye certain foreign substances may be detected. In pneumonokoniosis the sputum may show the particles of coal dust quite distinctly. In inhalation of iron dust the sputum may have a peculiar rusty color. Other foreign particles found in the sputum and due to inhalation may be seen in the sputum, such as the fine dust of flouring mills found in millers and the shining particles of sandstone seen in "stone cutters' phthisis."

Fibrinous tubes or casts of the bronchial tubes are occasionally seen in acute and chronic fibrinous bronchitis. These casts are best seen by spreading the sputum in water on a glass plate with a dark background.

Small casts of the smallest bronchi are often found in asthma, less commonly in acute pneumonia, constituting the asthmatic "spirals." As these are very small it may be necessary to examine them under a low power of the microscope.

Charcot—Leyden Crystals: These are slight, bluish shining, elongated octahedrals, varying much in size from those visible to the naked eye down to those only visible to a 1-6 objective. They occur quite abundantly just before and after asthmatic attacks. But are also found less constantly in acute and chronic bronchitis and tuberculosis.

Microscopic Examination: Elastic threads are found in the sputum in gangrene and abscess of the lung, and also in tuberculosis. They present a double outline, and often present the alveolar structure of the lung.

Tubercle Bacillus: By far the most important diagnostic constituent of the sputum is the tubercle bacillus. This occurs most abundantly in small white clumps or in thin white scales in the sputum, though they may be found in any portion of it. They are narrow rods, straight or occasionally moderately bent, their length varying from two to four micromillimetres. On account of its small size it is difficult to see without special staining. It takes up the aniline dyes with difficulty and when once stained is very resisting to the action of bleaching agents. Upon these characteristics a special method of staining is based.

The specimen of sputum to be examined is placed on a glass cover-slip and spread thinly and evenly over its surface, taking if possible the small white clumps spoken of above for examination. After spreading it thinly and evenly it is passed three times through the flame of a Bunsen burner or a spirit lamp to fix it to the slide. One of the most convenient stains is the one devised by Gabbet.

Two solutions are used. The first one consists of one part of fuchsine in 100 parts of a 5% solution of carbolic acid and 10 parts of absolute alcohol. The second solution is made by dissolving two parts of methylene blue in 100 parts of a 25% solution of sulphuric acid. The glass slip prepared as above is boiled in the fuchsine solution for two minutes, then placed in the methylene-blue solution for one minute, rinsed in water, dried, mounted in balsam and examined under the 1-12 immersive lens. The tubercle bacilli in these preparations are stained a bright red, the rest of the cells and bacteria being stained a deep blue. Only two other bacteria stain in this way; the leprosy bacillus and the smegma bacillus. In staining secretions for tubercle bacilli, where there is any chance for contamination by these bacteria, they may be differentiated by decolorizing for ten minutes in absolute alcohol after the carbol-fuchsine stain. By decolorizing with absolute alcohol for ten minutes the stain is removed completely from the smegma bacillus, while the stain of the tubercle bacillus is unaffected. The leprosy bacillus must be differentiated by cultivation experiments, but can usually be excluded by the clinical history of the case.

Pneumococcus: This coccus is the cause of croupous pneumonia, is found also in many of the complications of pneumonia such as empyema and meningitis. It occurs in the buccal secretions of perfectly normal, healthy individuals occasionally. It is easily stained with any of the aniline dyes. It is a capsule coccus, one, two or three occuring in a capsule, which is elongated or oval. Sometimes it occurs in short chains. It may also grow without the capsule, the form usually seen in cultures not showing the capsule.

Micro-cocci, bacteria and spirillæ of various kinds are often found in the sputum. They are much increased in fetid bronchitis, in bronchiectases, in abcess and gangrene of the lung, They are most conveniently stained in aqueous methylene-blue. after preparation of a cover-slip preparation as above.

EXAMINATION OF DISCHARGES.

The discharges with which we are most concerned are purulent discharges from cavities, or from some of the natural outlets of the body. The common bacteria of suppuration, the staphylo-coccus and strepto-coccus are readily stained by any of the aniline dyes, one of the most distinct being aqueous solution of methylene blue. Their morphological and culture characteristics will be taught by the bacteriologist and are only referred to here.

The gono-coccus is the only certain diagnostic point in gonorrhoea. A small drop of the pus is spread evenly on a cover glass, passed through a flame a few times and allowed to stand in aqueous methylene-blue solution for a few minutes. This is washed off and dried, finally mounting in balsam. The gono-coccus appears under the microscope as diplococci, shaped like a coffee bean, with the flat sides in opposition.

The gono-cocci may be found free between the cells, in the epithelial cells, and in the pus cells. The position *inside* the pus cells alone is characteristic as the psuedo-gonococcus occurs free and in the epithelial cells, and can not be distinguished from it by size or form. In cases of chronic gonorrhoea it often happens that the discharge stops and the patient is apparently well. Careful examination of the urine in these cases often shows a large number of pus-cells, and a few structures resembling small bits of thread. If one of these "mucous threads" be examined under the microscope it will be seen to consist almost entirely of pus cells, held together by a small amount of clear glassy mucous. If stained with methylene-blue and carefully examined under the 1-12 objective these will usually show a few gonococci.

STOMACH EXAMINATION.

In undertaking a chemical examination of the stomach contents, it is necessary to employ some standard meal or meals, whose rate of absorption, digestion and expulsion from the stomach into the intestine, has been carefully worked out by observation and experiment on normal individuals.

We employ in our work two meals given in combination, the Salzer meal and the Ewald breakfast. The Boas oat-meal gruel which will be spoken of more in detail under the head of lactic acid is given in special cases. For routine work we use the Salzer meal and Ewald breakfast as follows: At 8 A. M. we give the Salzer meal, compound of one soft boiled egg, about 60 grammes of rice, 30 grammes of lean, cold roast beef, cut or hashed into small bits and about 250 cc of milk. Exactly four hours after this (12 M.) we give the Ewald breakfast composed of one roll without crust (35 to 70 grammes) and 300—400 cc of warm water.

After one hour we withdraw the contents of the stomach by means of a small stomach tube, receiving the contents as they flow through the tube in a small clean basin, carefully avoiding any admixture of saliva with the contents.

The first examination is macroscopic and much can be learned of the condition of the stomach from this.

1. Appearance: The stomach contents should be clear, almost colorless, and contain only particles of partially dissolved bread from the Ewald breakfast. Particles from the Salzer meal at once indicate either a disturbance of the secretory or motor function of the stomach.

If a large amount of rice is found in addition to an excessive amount of the bread from the Ewald meal it usually indicates *hyperacidity* with faulty digestion of the starches.

If on the other hand we find some amount of proteid material left from the Salzer meal such as particles of egg, meat or coagulated milk, we may at once conclude that there is *subacidity* or *anacidity.*

Consistency: Normally the contents are quite fluid, flowing freely. They may be quite thick and ropy from the presence of an excessive amount of mucous. There may also be an excessive amount of mucous derived from the pharynx. This is easily recognized by the fact that the mucous is in clumps, and often, colored yellowish, greenish, and if in persons exposed to dust of a dark color. True stomach mucous is usually quite clear and spread uniformly through the contents.

Smell: Normal gastric juice has a distinctly sour smell not unpleasant. Under abnormal conditions the fatty acids may be formed by fermentative changes which give a very decidedly offensive odor to the contents.

Quantity: If possible to do so without injury to the patient, we may get the entire contents of the stomach which should not exceed 40 to 50 cc. If a very large amount 200 cc or more of a milky chyme-like fluid we may suspect some motor disturbance.

Blood: Blood may be present as fresh unchanged blood or it may be black almost like coffee grounds due to the action of the stomach fluids upon it. If at all doubtful we may test any suspected particles by the Turpentine—Guaiac test or the Haemin test as given under urinary analysis.

Bile: We can detect bile if present in large amounts by the color. If present in small amount only it may be recognized by any of the tests for bile pigment as given under urinary analyssis.

Microscopical Examination: Allow the stomach contents to stand in a conical glass for a short time. We may then take for examination portions from each layer of the contents and examine either stained or unstained specimens. The examination has reference to portions of the mucosa or the deeper layers of the stomach, to possible presence of plain muscular fibers. In neoplasms of the stomach after ulceration has commenced we may (rarely) find bits of the tumor which if large enough to show the arrangement of the cells may lead to a diagnosis of the disease. Probably a very few leucocytes might be found even in a normal stomach, but if numerous are due to a gastritis. In rare cases of phlegmonous or diptheritic gastritis, or in rupture of an abscess from the surrounding organs into the stomach, pus may be present in sufficient quantity to be visible to the naked eye.

Red corpuscles may be found in haemmorrhage into the stomach unless it has remained too long in contact with the acids of the stomach.

In cases of fermentation the frothy layer which rises to the top of the glass should be examined for bacteria. Under these circumstances we may find a number of kinds of bacteria, one of the commonest is called the sarcinae or wool-pack due to arrangements in *fours*.

CHEMICAL EXAMINATION.

After carefully filtering until the filtrate is clear we commence the chemical examination of the filtrate.

Reaction: Try first with blue litmus paper. If this is unchanged try again with red litmus which turns blue if the stomach contents are alkaline which occurs only in some cases of acute gastritis, in severe chronic gastritis and in cases where there has been a regurgitation of alkaline bile and intestinal juice from the small intestine. If the blue paper is promptly reddened the reaction is acid and the next step is to ascertain the acid or acids to which the acidity is due. We first dip a strip of congo paper into the filtrate. This paper is prepared by soaking filter paper for an hour in a 1% aqueous solution of congo-red and allowing it to dry. In the presence of free acids this paper turns blue but does not react to acid salts. If hydrochloric

acid is present the paper turns a dark blue not fading on the application of heat. Lactic acid turns the paper a dirty brownish blue which fades almost entirely when the paper is dried.

Tests for free HCl: The simplest and most convenient test for the presence of free HCl is the indicator called Dimethyl-amido-azo-benzol. A ½% alcoholic solution is prepared and kept in stock. A test paper is prepared by soaking filter paper in this for an hour and then drying.

This indicator or paper turns red or pink when it is dropped or dipped into stomach contents containing free HCl.

Boas' Test: Prepare a solution of resorcin resublimated five grammes, white sugar three grammes and 94% alcoholic 100 cc. Mix three drops of this reagent and six drops of the filtered gastric juice on a round white butter dish and heat gently over a water bath or open flame. In the presence of free HCl a bright red ring or mirror will form as evaporation goes on, fading in a few minutes. If HCl is absent only a faint yellow stain will result, and if heated too rapidly the residue will char, giving a black residue.

Gunzberg's Test: This solution is prepared by dissolving 2 grammes of phloroglucin and one gramme of vanillin in 30 cc of absolute alcohol. A yellow liquid results which gradually turns red and finally brown on an exposure to the light. This solution must then be kept in a dark bottle away from the light. This solution is used in exactly the same way as the Boas test given above.

Lactic Acid: Lactic acid is present in traces in most of the test meals. It is formed by the action of certain bacteria of the mouth upon carbohydrates and is found in even healthy stomachs for some time after the ingestion of the meal, the curve of lactic acid acidity falling as the curve of HCl acidity rises until at the height of digestion no lactic acid should be demonstrable by ordinary tests. This fall in the amount of lactic acid is due to two factors; firstly the absorption of the preformed lactic acid secondly the antiseptic action of the HCl of the gastric juice inhibiting or destroying the bacillus which causes the acid fermentation. Many complicated tests have been devised for the detection of lactic acid but the most convenient one for clinical purposes is that of Uffelman.

Test: A solution containing three drops of a strong solution of Ferric chloride and 3 drops of carbolic acid in 20 cc of water. This solution is a bright amethyst blue and should be translucent when placed in a test tube. To this solution add a few drops of the stomach filtrate which in the presence of lactic acid will give a bright canary yellow color. In some cases it is convenient to make a layer test by placing a small amount of the filtrate in the bottom of a test tube and floating the solution upon it. Hydrochloric acid decolorizes the solution and butric acid gives a faint greenish-yellow color to the solution.

The persistent and excessive formation of lactic acid is stated to be quite characteristic of cancer of the stomach. In suspected cases therefore it is necessary to give some test meal which contains *no* lactic acid. Such a test meal is furnished by the Boas test meal.

This consists of a tablespoonful of rolled oats boiled in a 1000 cc of water until it measures 500 cc. This may be salted a little but nothing else added.

The stomach is washed out thoroughly the night before the exhibition of this meal, the oatmeal gruel given in the morning and withdrawn again in one hour. The presence of an amount of lactic acid which can be demonstrated by the Uffelman test in conjunction with other symptoms renders the diagnosis of gastric cancer probable.

Butyric Acid: Can usually be detected by its odor, that of rancid butter.

Acetic Acid: This acid can usually be detected by its odor also. It may be demonstrated in the following manner.

Ten cc of the filtrate is extracted with ether, the ethereal extract evaporated after separation, the residue dissolved in a few drops of water and exactly neutralized with a dilute solution of caustic soda. If a drop of a very weak solution of perchloride of iron be added to this a deep red color results in the presence of acitic acid.

Determination of Amount of Free HCl: The determination of the amount of free HCl is most conveniently done by the use of the indicator spoken of under tests for free HCl, viz: Dimethyl-amido-azo-benzol. Five or 10 cc of the filtered gastric juice is diluted with distilled water, a few drops (2 or 3) of the indicator added. In the presence of free HCl a red color develops. Carefully add a decinormal solution of caustic soda until the red color disappears and the solution becomes a clear lemon yellow which indicates that all the free HCl has been neuralized. The *degree* of acidity is found as follows: 10 cc of the filtrate required 1.5 of the decinormal solution of caustic soda to neutralize it and 100 cc would require 10 times 1.5 to neutralize it giving therefore 15 degrees of acidity due to free HCl. The percentage of HCl is found in a similar manner. Each cc of the soda solution will neutralize 0.00365 grammes of HCl, therefore the 10 cc contained $0.00365 \times 1.5 = 0.005475$ grammes of free HCl each cc contained 0.0005475 grammes the percentage being 0.05475.

Method of Mintz: 10 cc of the stomach contents are treated with a decinormal caustic soda solution until they give no reaction with the Boas or Gunzberg reagents. Allowing for the loss due to a portion of the gastric juice being taken out for the Boas or Gunzberg test this gives a very reliable test for the HCl. The degrees of acidity and the percentage of HCl are found as in the previous method.

Still quicker and easier is a method suggested to me by Dr. Hemmeter. A gastric juice containing only a small amount or

none of the fatty acids and lactic acid, is titrated with decinormal caustic soda solution until a small drop taken out with a platinum wire and dropped on a slip of congo-paper does not change the color of the paper. In the presence of large amounts of lactic acid a dirty brownish blue discoloration of the paper will take place, even after the free HCl has been neutralized, so that great care must be exercised in these cases.

Estimation of the Fatty Acids: Take the total acidity of a given quantity of the gastric juice. Another portion of the same amount exactly is evaporated on a water bath to a *syrupy consistency* and made up to the same volume again with distilled water. The evaporation has driven off the fatty acids and by taking the total acidity of this solution we may get the acidity due to the fatty acids by finding the difference between the first titration and the second determination.

The acidity due to organic acids may be determined in still another manner. 10 cc of the filtered gastric juice is exactly neutralized with decinormal caustic soda solution. This is then evaporated to dryness and the residue burned, being careful not to carry the incineration further than the point at which the residue burns with a luminous flame. If carried beyond this point some of the chlorides are liable to be volatilized and lost. Dissolve the residue in 10 cc of water and titrate with decinormal sulphuric acid.

By incineration all the organic acids were converted into carbonates of the alkali used. These are titrated with decinormal sulphuric acid, and the amount of acid used will represent the amount of organic acids, which may be most conveniently expressed in terms of HCl.

Estimation of Free HCl, Combined HCl, and Acid Salts, Organic Acids, Etc. The most convenient method of estimating the various forms of acid to which the total acidity of the stomach is due is that of Töpfer. In this method three different indicators are used.

1) 0.5% alcoholic solution of dimethyl-amido-azo-benzol.
2) 1% aqueous solution of alizarine.
3) 1% alcoholic solution of phenol phthalein.

In practice 5 or 10 cc of gastric juice is measured out into three beakers. A few drops of phenol phthalein and a few cc of distilled water are added to this and the mixture titrated with a deci-normal caustic soda solution until the rose color that appears when the neutral point has been nearly reached has deepened into a dark red. This color indicates that all the various acidities of the gastric juice have been saturated, viz.: the free HCl, the combined HCl, the acid salts and the organic acids.

To the second beaker three or four drops of the aqueous solution of alizarine are added and the solution titrated with the caustic soda solution until a purple color appears. The appearance of this purple tint indicates that the free HCl, the acid salts and organic acids have been saturated, but *not* the combined HCl.

The difference between No. 1 and No. 2 therefore will be the amount of HCl in combination with albuminous matters of the food, the so-called "combined HCl," which has been secreted by the stomach and already performed its physiological functions in digestion.

To the third beaker three or four drops of dimethyl-amido-azo-benzol solution are added. In the presence of free HCl, a decided red tint develops, but if absent only a yellow color will be found. If a red color, denoting the presence of free HCl appears, titrate with the caustic soda solution until the red color changes to a bright lemon yellow. The amount of NaOH needed to produce this change in color is the amount necessary to neutralize the free HCl of the gastric filtrate. The difference between the amount required to produce the purple color with alizarine and the amount necessary to produce the yellow color with the dimethyl-amido-azo-benzol will represent the amount of NaoH required to neutralize the acid salts and the organic acids of the filtrate.

In stating the results of these determinations we may employ two methods. First, by stating the amount of NaOH in cc to neutralize each acidity in 100 cc of the filtrate as so many "*degrees*" of acidity. Second, by calculating each acidity into its equivalent in HCl for one cc, remembering that each cc of decinormal caustic soda solution will neutralize 0.00365 grammes of HCl.

Winter's Method: This method is much longer and more complicated than the preceding method, but is interesting as a comparative method. Five cc of the filtered gastric juice are placed in each of three porcelain capsules and numbered one, two and three.

No. 1 is evaporated to dryness and the residue burned until the residue ceases to burn with a luminous flame. By this treatment the free HCl and the combined HCl are driven off, leaving only the Cl in combination with the inorganic bases. After cooling, the contents of the capsule are treated with a few drops of nitric acid and about five cc of distilled water, carefully stirred with a glass rod, neutralized by adding an excess of calcium carbonate, and filtered through a dry filter, which is carefully washed with distilled water until free from chlorine.

No. 2 is evaporated down just to dryness, being careful to avoid burning, made up to 5 cc again with distilled water, an excess of pure sodium carbonate added and finally evaporated, incinerated the further treatment being the same as No. 1.

No. 3 is treated immediately with an excess of sodium carbonate, evaporated, incinerated and treated exactly as No. 1.

By a study of the methods used it will be seen that No. 1 contains the chlorine which was contained in the filtrate in combination with the inorganic bases. No. 2 contains the chlorine in the inorganic bases and the Cl in combination with the proteid bodies, the combined HCl, while No. 3 will contain the total chlorine, consisting of inorganic chlorides, combined and free HCl.

The amount of chlorine in each filtrate is then estimated as in the estimation of chlorides in the urine.

The filtrate and the washings of the filter are placed in a flask, and to it is added about 4 cc of pure nitric acid of 1.200 Sp. Gr. Add also 5cc of a saturated solution of ferric ammonium alum and 15 cc of the standard silver solution. Titrate the mixture with the ammonium sulpho cyanate solution until a red color appears, due to the formation of the sulphocyanate of iron.

CALCULATION: As in the absence of chlorides 37.5 cc of the ammonium sulphocyanate solution would be required to neutralize the 15 cc of silver solution, the difference between 37.5 cc and the number of cc actually required, represents the amount of silver nitrate used up in the saturation of the chlorides.

Then as each cc of the ammonium sulphocyanate solution represents .004 grammes of Na Cl, the amount of chlorine, estimated as Na Cl, in the 5 cc of filtrate is found by multiplying the difference between 37.5 and the actual amount used by .004. The amount of chlorine may be found by the following formula, x being the number of grammes of Cl estimated as Na Cl:

58.4 : 35.4 :: x : ( ).

TOTAL ACIDITY.

For many cases the determination of the total acidity is sufficient for diagnostic purposes. A small amount of the gastric juice 2.5 or 10 cc is diluted with a small amount of distilled water, and a few drops of a 1% alcoholic solution of phenolphthalein added to it. Titrate carefully with a deci-normal caustic soda solution until a dark red color appears, denoting a faint degree of alkalinity of the mixture. The total acidity is expressed as so many "degrees" of acidity.

For example, 5 cc of the filtrate required 2 cc of the caustic soda solution to neutralize it. Then if 100 cc of the filtrate had been taken 40 cc of the caustic soda solution would have been required to neutralize it, or it is more simply stated as 40 degrees of total acidity. In general terms it may be stated that after the double test meal of Salzer the normal stomach shows an acidity of from 40 to 60 degrees. The total acidity is increased usually in ulcer, in early stages of cancer developing on the base of an old ulcer, and in neuroses to which the general term *hyperacidity* has been applied.

The degree of acidity is decreased as a rule in acute and chronic inflammatory diseases of the stomach, in carcinoma, and in many of the neuroses. A neutral or alkaline reaction is rare, most frequently occurring in chronic mucous gastritis, in prolonged vomiting where some of the intestinal contents are forced into the stomach and ejected by vomiting, and in some cases of "morning vomiting" in which the vomited matter consists mainly of saliva which has been swallowed during the period of nausea preceding the act of vomiting.

ERYTHRODEXTRIN: Ptyalin, the digestive ferment of the saliva, acts exclusively upon the starches, acting readily upon boiled starch, acting only slightly on raw starch. The end product of its action is maltose, a number of dextrins being formed as intermediate products. This action only takes place in an alkaline or very faintly acid medium, even a moderate degree of acidity checking its activity. The first dextrin formed is erythrodextrin which gives a purple color with a dilute solution of iodine or a weak Lugol's solution. Achrodextrin the next product is nearly colorless, giving only a faint reddish tinge with Lugol's solution, while maltose, the final product is colorless.

In the normal stomach, this action of the ptyalin continues for some little time, probably 15 or 20 minutes before the acidity of the gastric juice is raised to a degree sufficient to check the action of the ptyalin, and in this time the starch is mainly transformed into maltose and achroodextrin.

On the other hand in cases of hypersecretion and hyperacidity the gastric juice reaches a degree of acidity sufficient to stop the action of the ptyalin before the starch is all transformed, and as a result we get the purple reaction of erythrodextrin. This test is of value as confirmatory evidence of hyperacidity.

EXAMINATION FOR PEPSIN: Unfortunately we have no exact quantitative tests for pepsin so all tests must be relative. Pepsin is probably not secreted as such by the peptic glands but in its stead an enzyme or mother substance is secreted which is changed into pepsin by the acids of the stomach. This mother substance is called pepsinogen. The change from pepsinogen to pepsin is best performed by hydrochloric acid, but in the absence of hydrochloric acid the same change is affected by the organic acids but less perfectly.

Pepsin is best tested for by its action on egg-white. A small bit of egg-white .5 cm square by about 1 mm in thickness is placed in a test tube with 3 cc of the gastric juice and kept at a temperature of from 98° to 100° F for two hours. If Pepsin is present in normal amount the egg should be dissolved within this time.

PEPSINOGEN: In case the gastric juice is alkaline, neutral or show no trace of free HCl only organic acids being present, it is necessary to test for pepsinogen. Prepare three test tubes, placing in each 3 cc of the gastric juice and the small bit of egg-white as above. The first one is put in the warm oven without any addition to it. To the second 0.5 cc of a 1% solution of hydrochloric acid is added, and to the third 0.5 cc of a 1% solution of hydrochloric acid, and a grain or two of pure pepsin and placed in the warm oven at a temperature of 98° to 100° and kept there for two hours.

At the end of this time if all three show digestion of the egg, pepsin is present. If only No. 2 and No. 3 show digestion of the egg pepsin is absent and pepsinogen present, if only No. 3 shows digestion of the egg, pepsin and pepsinogen are both absent.

If digestion takes place in the tubes to which pepsin has

not been added, but requires longer than two hours, pepsin or pepsinogen as the case may be is present but in diminished amount, indicating a decrease in the digestive powers.

Rennet Ferment: Place 3 cc of good milk with a nearly neutral reaction in a test tube and add to it 3 drops of the gastric juice. Place it in the warm oven at the body temperature, where coagulation will take place in from 5 to 15 minutes should rennet ferment be present in normal amount.

Rennet Zymogen: In the absence of free HCl, rennet zymogen may be present in normal amount while rennet ferment would be absent by the above test. It is tested for by exactly neutralizing about 3 cc of the gastric juice and adding a few drops in excess of caustic soda solution. Add to this 1 cc of a 1% solution of calcium chloride and 4 cc of neutral milk and place in the warm bath or oven for a few minutes. Coagulation should take place in from 5 to 15 minutes.

Of considerable diagnostic and prognostic value is the quantitative estimation of rennet zymogen. The secretion of HCl may be disturbed by many causes, motor and nervous, while rennet zymogen seems to suffer the least from these disturbances. Prepare several dilutions of the gastric juice 1:10, 1:20, 1:30, 1:50, 1:75, 1:100 and 1:150. Take 3 cc of each of these dilutions, faintly alkalinize them with caustic soda solution, and put in separate marked test tubes. Add to each tube 1 cc of a 1% calcium chloride solution and 4 cc of milk and place in the warm oven for 15 minutes. At the end of this time notice the dilution at which coagulation has taken place. In the normal stomach, a dilution of from 1:100 to 1:150 should be active. In cases of gastritis the filtrate is active only in dilutions of 1:20 to 1:40. A gastric juice which is active only in dilutions below 1:20 is probably due to an atrophic gastritis, the prognosis with regard to a cure being very bad. From 1:20 to 1:40 the prognosis is fairly good.

Test for Absorption: A gelatine capsule containing 0.2 grammes of potassium iodide is given to the patient, shortly before a meal and the saliva examined for the presence of iodine at intervals of from 2 to 3 minutes. The most convenient method of conducting this examination is by means of starch paper and fuming nitric acid. The starch paper is prepared by soaking unglazed paper in boiled starch and allowing it to dry. When wanted for use moisten with a little water. The paper is touched with a rod dipped in the saliva or a small drop of the saliva dropped upon it. With a glass rod put a small drop of fuming nitric acid on this. A purple color is produced when the iodine makes its appearance in the saliva, which takes place in a normal case in from 6 to 11 minutes.

Motility: The best practical test for motility is the examination of the contents of the stomach after the double test meal of Salzer. In the normal stomach there should be no trace of the Salzer meal itself. Simply a small amount of half-dissolved bread in a clear or faintly cloudy liquid. If there is meat, etc., there

probably is a deficient motor power of the stomach. In some cases where dilatation and atony is suspected it is advisable to withdraw the contents of the stomach in the morning before eating. In cases of dilatation and atony of a severe grade it is usual to find traces of the previous meal, taken the previous night, at least 12 hours before the examination.

Ewald's Test: One gramme of salol in a capsule is given the the patient immediately after the breakfast or dinner and the patient directed to pass his urine at intervals of half an hour for two hours and save each portion separately. To a small portion of each a drop of a weak solution of ferric chloride is added which in the presence of salicyluric acid will give a purple tint.

This test is based upon the fact that salol is broken up in an alkaline medium into carbolic and salicylic acid. Except in the presence of a large amount of alkaline mucous, this decomposition does not take place in the stomach, but on the other hand may not take place in the intestine, due to acid fermentation in the intestinal contents.

Under normal circumstances the reaction may be demonstrated in the urine in from 45 to 75 minutes, being delayed in cases of atony to 2 hours or longer. A continued presence of the reaction up to 24 hours or longer is also indicative of obstruction or atony. It must be remembered also that in some cases of acute gastritis, all these tests may indicate serious disease, but that after a few days, recovery may take place and the stomach be found perfectly normal. It is necessary therefore to make at least three examinations before a definite diagnosis and prognosis can be made.

Examination of the Fasting Stomach: In health the stomach secretes continuously a small amount of juice containing usually a small amount of HCl, pepsin and rennet ferment. A variable amount may be obtained by expression, usually only a few cc. As much as 50 cc may be obtained in some cases from an apparently normal stomach. A secretion of over 50 cc points to a condition called peptonorrhoea, and may be combined with hyperacidity, a condition known as continuous hypersecretion and hyperacidity.

EXAMINATION OF FAECES.

QUANTITY OF FAECES: Assuming that the gastro-intestinal tract is unobstructed, the amount of faeces depends on the quantity and quality of food taken. It is easily seen that an increase in the amount of matter ingested and the ingestion of a large proportion of vegetable matter will lead to an increase as this latter contains a larger proportion of indigestible matter which cannot be absorbed. The quantity of faeces is increased in diarrhœa, due either to a quickened peristalsis or to an effusion from the intestinal mucosa. After constipation, there may be a large amount of firm hard faecal matter, this increased excretion persisting for some days. In faulty absorption due to disease of the liver, pancreas, mesenteric glands or intestinal mucosa the amount of faecal matter is increased.

CONSISTENCY AND FORM OF STOOL: The normal stool is firm or mushy. In diarrhœa the stool is thin, watery or like thin soup, due both to the increased peristalsis, or to insufficient absorption and in some cases to the exudation of fluid from the intestinal mucosa as in Asiatic cholera, severe cases of acute enteritis, dysentery and typhoid fever. The form of firm faeces has litttle diagnostic value, though a flat ribbon-like stool may indicate stenosis of the rectum and is seen in severe cases of prostatic hypertrophy.

ODOR: The variations in odor from the normal often have diagnostic value. An odor of butyric acid with a slight acid reaction is seen in some cases of infantile diarrhœa with acid fermentation. A decidedly foul smell as of some decomposing animal substance is seen in more severe cases of this affection. When there is a considerable mixture with blood the faecal odor may be masked completely by the slightly unpleasant odor of blood. The stools of gangrenous dysentery, carcinomatous or syphilitic ulceration of the rectum often have foul cadaverous odor.

REACTION: Normally the reaction is nearly neutral, in nursing children faintly acid.

It may be distinctly acid in acid fermentation, or distinctly alkaline in alkaline fermentation, in either case being due to a catarrhal condition of the intestinal tract.

COLOR: The normal color is brown, varying in tint. This color is due to decomposition products of the biliary coloring matters. The stool may be colored by articles of food or by drugs. Iron colors the stool nearly black, bismuth a greyish-black. In obstructive jaundice the stool is usually mushy, greyish-white in color, often faintly glistening due to the presence of fat. Bilious stools on the other hand are those in which, due to rapid peris-

talsis, the bile pigments have not had time to undergo complete transformation. This may be demonstrated by filtering the faeces and testing the filtrate for bile pigments as given under urinary analysis.

Foreign Particles: In the normal stool portions of food can be recognized with the unaided eye if indigestible particles have been eaten, such as the seeds and skins of various fruits. Large fibres of connective tissue, undigested portions of grains, etc., may be met with in the stools if the food has been eaten rapidly or in large quantities. Muscular tissue, clumps of casein and bits of starch may be seen in the stool in cases of catarrh of the stomach or small intestine or in the catarrh and rapid peristalsis of some kinds of fever.

Mucus: Mucus visible to the naked eye always indicates a catarrh of the mcus membrane of the intestine. Mucus occurring in solid fecal balls, in a fine equal division, usually means catarrh of the small intestine, it may also occur as a fine intimate mixture in thin stools in cases in which the large intestine is also affected. Large masses of mucus in more or less thick shreds, or firm faeces covered with a layer of mucus usually indicate catarrh of the large intestine.

Purulent Stools: A considerable collection of pure pus is not infrequently found in the stool, due to the rupture of a collection of pus into the bowel. This usually comes from some pelvic abscess or may come from the rupture of the pus from an appendicitis into the bowel.

Usually this is easily recognized by the naked eye but if necessary and suspicious a portion should be examined microscopically.

Dysenteric, syphilitic and carcinomatous ulcerations of the large intestine produce more or less pus depending upon their extent, and this may be in amount sufficient to be recognized with the unaided eye.

Gall Stones: These come either from the gall-bladder or from the hepatic ducts and usually cause severe colic and often jaundice. After every case of severe abdominal pain, especially if followed by jaundice, the stools should be examined for gall-stones. This is best done by passing the stool through a sieve, if formed or mushy, breaking it up in water before passing through the sieve. Some conclusions may be drawn from the size and shape of the stone as to its source. Those from the gall-bladder are usually of some size and often present facets and many angles produced by other stones in the gall-bladder. Stones from the hepatic ducts are usually of small size, crumbling easily, and rarely shows facets or angles, usually being rounded.

Portions of Tissue: In intussusception, the entire intestine invaginates and if life be prolonged sufficiently, the invaginated portion may come away in the stool, effecting a spontaneous cure. Shreds of tissue in ulcerations, or bits of tissue from new formations, as carcinomata, may be found in the stools.

Tape-worm: This worm consists of a very small head and neck and a ribbon of flat joints, often several metres in length. It inhabits exclusively the small intestine, clinging to the mucous membrane with its head and growing from above downward, the last joints dropping off as growth goes on.

It can be recognized by a single joint, or it may be recognized by the passage of eggs.

There are three varieties of the tape-worm which are commonly met with in the intestine.

Taenia Solium: This is from 2 to 3 metres in length, the head, gray in color about the size of the head of a pin. The joints on the lower part of the worm are yellowish-white in color and measure about 10 mm in length by 5 to 6 mm in breadth, resembling a gourd seed. These ripe joints are thrown off frequently in the stools and from this peculiarity as well as the formation of a longitudinal canal (the uterus) we are able to differentiate it from the other varieties of tape-worm. This canal gives off as many as a dozen branches from its sides which subdivide like the branches of a tree. The eggs are round with a hard shell requiring a moderate magnifying power for their detection. The head when examined under a low power will show a circular row of hooks srurounded by four pigmented sucking discs or cups.

Taenia Mediocanellata grows to a length of 4 or 5 metres. Its head is larger than that of taenia solium and has no zone of hooklets, only showing the four sucking cups which are larger and stronger than those of taenia solium.

The ripe segments are passed by the stool and also wander from the anus independently, having a free movement of their own. They are slightly larger and thicker than those of taenia solium.

Bothriocephalus latus is the largest of the tape-worms reaching a length of 7 to 8 metres. Its head is long, narrow, with two long narrow sucking cups. The joints are not passed singly but a large fragment comes away at long intervals. The eggs are quite large with a thin brownish capsule, provided with an opening on one end, covered by a cap of the same material.

Ascaris Lumbricoides: These are easily recognized by their resemblance to the common ground worm. They inhabit the small intestine, but have been found in vomited matters, in the ductus communis choledochus as the cause of jaundice, they are found in the stools and occasionally crawl out of the anus during sleep.

Oxyuris Vermicularis: This is a small white worm resembling very much a bit of white thread, inhabiting especially the large intestine, caecum and rectum and occasionally wandering into the vagina.

Trichina Spiralis: The worm is rarely seen in the intestinal contents but may occur there in the first stages of trichi-

nosis. They are only one-third the size of the oxyuris and must be examined for with a low power magnifying glass. In later stages of the disease the worms become encysted in the muscles. A small bit of the muscle may be cut out, teased and examined for the parasite.

Infusoria of many kinds occur in the intestinal contents, usually associated with acute or chronic catarrh, &c. Most of them are of no clinical significance whatever.

Amoeba Coli: This amoeba has been found to be the cause of a special form of dysentery. The suspected stool should be kept at body temperature and examined as soon as possible after its passage. A small bit of the white mucous is taken and placed on a clean, warm slide, covered with a thin cover-glass, pressed down to make a thin uuiform layer and examined under the 1-5 objective. The amoeba is seen as a round body, freely movable, varying in diameter from 20 to 40 micromillimetres. It contains a small clear body in its center called the endosarc surrounded by a zone of more granular material the ectosarc. If kept warm and carefully watched the amoeba will be seen to throw out a projection called a pseuedopodium and soon the entire body of the parasite will be seen to have moved into the psuedopodium. This parasite should always be looked for in the pus from abscesses of the liver, especially if at any time there had been symptoms of dysentery preceeding it.

VEGETABLE PARASITES.

Many forms of vegetable bacteria occur in the intestinal tract, whose identification can only be done by culture characteristics, Probably the most important bacillus for the physician is the bacillus of tuberculosis. This is stained for and examined as given under examination for sputum. If the bacillus is present in repeated examinations and is associated with symptoms of diarrhœa, etc., the existence of intestinal tuberculosis may be inferred, though not definitely, as the bacilli may come from sputum which has been swallowed. In many of these cases there is no appearance of pus in the stool and a hap-hazard hunt is usually negative. In such cases the bacillus may be demonstrated by taking a small bit of the mucous from the mucous membrane of the rectum and subjecting it to the stains for the tubercle bacillus. In all cases of chronic fistula-in-ano, with or without definite tuberculosis of other organs of the body, the pus from the fistula should be examined for the presence of the tubercle bacillus. If present this will either call for non-interference if the disease is extensive and if not extensive will call for a more radical operation and a more guarded prognosis than the ordinary non-specific fistula-in-ano.

PARASITES OF THE SKIN.

VEGETABLE.

Achorion Schonleinii: This fungus occurs in the root-sheaths, bulbs, hairs near the skin and in the crusts of the different varieties of favus. The crusts or roots of the diseased hairs are placed in a little water or in dilute liquor potassæ and allowed to macerate for a short time, after which they may be examined without further preparation, or may be stained with aqueous methylene blue and examined under the 1-6 objective. It is seen to consist of dense masses of mycelium or threadlike material and a large number of round or oval bodies, the spores of the fungus. The threads or mycelium are jointed and the bodies between the joints are apparently divided into compartments.

Trichophyton Tonsurans: This fungus which is found in all the different varieties of tinea or ringworm resembles the preceding and is examined for in the same way. Usually it is less abundant, much less branched, shows fewer of the compartments spoken of under Achorion and the spores are less abundant. It is most abundant about the roots of the hair in the affected area and is most readily found by removing a few hairs from the affected area, softening the root and bulb with liquor potassæ and examining under the 1-6 objective.

Microsporon Furfur: The microsporon furfur is very easily found in the characteristic scales of tinea versicolor resembling quite closely the achorion but more prone to the development of spores, the latter being highly refractive and resembling minute globules of oil.

ANIMAL.

Practically in this climate the only animal parasite which needs the microscope for its detection is the itch mite or acarus scabiei. By close scrutiny in almost every case a burrow formed by the adult female may be found, the easiest place being on some covered portion of the body where it has not been altered by the scratching of the patient. A person with good eyesight can easily pass a fine cambric needle along and a little beyond the end of the burrow and uncover the insect. By the naked eye this can be recognized as a distinct yellowish white dot, quite distinct from the blackish excrement filling the rest of the burrow. The insect may be lifted from its burrow on the point of the needle and placed under the microscope, where its body is seen to be of an oval form, with a short projecting head. Its dorsum is convex and barred transversely. Its ventral surface is flat and is provided with eight small claws or legs. The males are smaller, resemble the female more or less but do not burrow in the skin.